UNIVERSITY OF BRISTOL

THE ANNUAL REPORT

OF THE

Agricultural and Horticultural Research Station

(THE NATIONAL FRUIT AND CIDER INSTITUTE)

LONG ASHTON, BRISTOL
1952

BATH :
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H. C. BUTTERFIELD, M.A. (Com.).

LIST OF STAFF—1952.

Long Ashton Research Station.

Director :
Professor T. Wallace, C.B.E., M.C., D.Sc., F.R.I.C., V.M.H.

Secretary :
A. L. Leggett, A.S.A.A.

Nutrition of Fruit Plants :
C. Bould, M.Sc., Ph.D.
J. A. H. Tolhurst, B.Sc.
Miss M. P. Scadding (*Transferred to Entomology Section* 31 *July,* 1952).
Miss B. Hood (*Appointed* 8 *September,* 1952).

Pomology and Plant Breeding :
L. C. Luckwill, B.Sc., Ph.D.
G. T. Spinks, M.A., Dip. Agric. (Plant Breeding).
E. W. Hobbis, B.Sc., N.D.H. (Plantations Officer).
D. L. Abbott, B.Sc.
Miss H. M. Ferres, M.Sc. (*Resigned* 31 *January,* 1952).
R. R. Williams, B.Sc., Dip. Hort. (*Appointed* 10 *January,* 1952).
Miss B. A. Rake, B.Sc., N.D.H.
P. S. Weaver (*Appointed* 1 *February,* 1952).

Plant Physiology :
E. J. Hewitt, B.Sc., Ph.D., A.K.C. (*Seconded to A.R.C. Unit of Plant Nutrition* 1 *April,* 1952).

Chemistry of Insecticides and Fungicides :
J. T. Martin, B.Sc., Ph.D., D.Sc., F.R.I.C.
D. Woodcock, M.Sc., Ph.D., F.R.I.C. (Organic Chemistry).
W. D. E. Thomas, B.Sc., Ph.D., A.R.I.C. (Physical Chemistry).
*E. Somers, M.Sc.
†G. V. Coles, B.Sc. (*Appointed* 4 *February,* 1952).
C. P. Lloyd-Jones, B.Sc., A.R.I.C. (*Appointed* 11 *August,* 1952).
R. F. Batt.
Miss P. M. James.
A. J. Sims.
J. F. Harris (*Appointed* 1 *September,* 1952).

Entomology :
H. G. H. Kearns, B.Sc., Ph.D., Dip. Agric.
S. H. Bennett, B.Sc.
N. G. Morgan, B.Sc.
A. Stringer, B.Sc., A.R.C.S.
A. H. Wilkins.
Miss E. B. Ward.
G. Bracey.
Miss M. P. Scadding (*Transferred from Fruit Plant Nutrition Section* 31 *July,* 1952).

*Special Colonial Office grant for studies of spray retention.
†Special grant from Rubber Research Institute of Malaya.

LIST OF STAFF—*continued.*

Plant Pathology :	R. W. Marsh, M.A. S. H. Crowdy, B.Sc., A.R.C.S. (*Resigned* 31 *May*, 1952). R. J. W. Byrde, B.Sc. (Hort.), Ph.D. A. T. K. Corke, B.Sc. (*Appointed* 1 *September*, 1952). A. H. Fielding. Miss P. M. Tozer. Miss M. Marlow.
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Domestic Food Preservation :	Miss B. A. Crang, B.Sc. Miss M. Sturdy, B.Sc. (H. & S.S.). Miss M. Johnstone, B.Sc. (H. & S.S.). Miss B. C. Hart. Miss M. M. Hooper.
Willows :	K. G. Stott, B.Sc. (*Returned from National Service* 16 *April*, 1952).
Technical Section :	H. S. D. Swan, M.A. (*Resigned* 26 *August*, 1952). M. Greenwood, O.B.E., M.Sc. (*Appointed* 20 *October*, 1952). G. E. Clothier (Station Guide and Meteorological Recorder).
Library :	G. T. Spinks, M.A. (Honorary Librarian). Miss I. E. Taylor.
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Photography :	G. H. Jones, A.R.P.S. E. R. Wilkins.
Plantations and Greenhouses :	E. W. Hobbis, B.Sc., N.D.H. (Plantations Officer). S. H. Legge (Marketing Officer and Recorder). E. Catlow (Recorder). A. I. Campbell, N.D.H. (Propagator and Recorder). R. M. Jarrett (Recorder). C. W. Harper (Spraying Recorder). D. W. Burd (Recorder) (*Appointed* 1 *October*, 1952). A. J. Hurdle (Plantations Foreman). H. Locke (Horticultural Assistant). Mrs. R. Lewis (Assistant Recorder). J. G. Clarke (Greenhouse Foreman).

LIST OF STAFF—*continued.*

Workshops and Maintenance :
S. E. Cole.
H. J. Jones.
B. F. Shearn.
L. W. French.

Canteen :
Mrs. N. M. King.

Administrative and Clerical Staff :
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D. H. Pittman (Cashier).
Miss M. H. Simpson (Director's Secretary).
J. Packer (Senior Clerk).
Miss E. M. Matthews.
Miss V. E. R. Alsop.
Mrs. J. Packer (*Resigned* 31 *March*, 1952).
Miss B. J. Haysham.
Miss M. A. Febrey (*Resigned* 9 *February*, 1952).
Miss N. K. Bullock.
T. J. Ford (*On National Service from* 20 *October*, 1952).
Miss B. E. Chapman (*Appointed* 3 *November*, 1952).

Outdoor and Cider House Staff with more than 20 years' service :

R. Jones	(30).
J. C. W. Barnes	(24).
W. M. Hynam	(24).
F. W. Hynam	(23).
K. J. Escott	(22).

Agricultural Research Council Unit of Plant Nutrition (Micro-Nutrients).

Honorary Director :
Professor T. Wallace, C.B.E., M.C., D.Sc., F.R.I.C., V.M.H.

E. J. Hewitt, B.Sc., Ph.D., A.K.C. (*Seconded to Unit*).
*D. J. D. Nicholas, B.Sc., Ph.D., A.K.C.
W. Plant, B.Sc. (Hort.), Ph.D., N.D.H.
A. H. Williams, M.A., B.Sc.
E. W. Bolle-Jones, M.Sc., Ph.D. (*Resigned* 6 *July*, 1952).
W. A. Forster, B.Sc.
C. C. McCready, M.A., D.Phil. (*Appointed* 17 *November*, 1952).
Mrs. A. H. James, B.Sc. (Hort.)—(Miss O. M. Woodhouse).
D. F. Day, B.Sc. (Hort.) (Greenhouse Experiments Officer) (*Appointed* 21 *January*, 1952).
W. J. Redmond.
D. J. Fisher.
D. G. Hallas.
B. A. Notton.
H. M. Webber.
Mrs. S. M. Taylor—(Miss M. V. Hallett).
M. A. Wright.
Miss R. A. Paget.
R. T. Dewell.
J. Jarrett.
Miss J. R. Brown.
Graham H. Jones.
M. W. J. Heyes.
Miss J. E. Mores.

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*From 18 September, 1952, temporarily attached to the McCollum Pratt Institute, Johns Hopkins University, Baltimore, Maryland, U.S.A., during the tenure of an E. J. Johnson Fellowship.

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INTRODUCTION.

By T. WALLACE, Director.

Nineteen-fifty-two has been notable as a year of steady progress in the main investigations of the research programme and for some important events and changes relating to the work and administration of the Station.

The Institute staff has retained its close liaison with the horticultural officers of the N.A.A.S. in the various ways described in previous reports. The annual refresher course for N.A.A.S. Horticultural Officers was held at the Station on July 16th and 17th, when lectures and demonstrations were given by members of the Institute staff. The special pruning course for N.A.A.S. Officers was also held under the joint supervision of Mr. F. A. Roach of the N.A.A.S. and Mr. Hobbis, the Station Plantations Officer.

The relationships between the Institute and the N.A.A.S. Horticulture Experimental Stations have been maintained. The Director has again acted as chairman of the Fruit Working Party for co-ordinating experiments at these stations and has also served on the Vegetable Working Party and on the Advisory Committees of a number of the Stations. Good progress can be reported in the fruit experiments at the Luddington Station, and arrangements have also been made to establish a number of fruit plots at the Efford Station, Lymington, Hants. The Director has also continued as chairman of the Fruit Group of the N.A.A.S., which is responsible for the preparation of the bulletins and leaflets on fruit culture published by the Ministry of Agriculture.

During the year a major change was made in the administration of the special work on the mineral nutrition of plants. From April 1st, 1952, the group of workers concerned were, by agreement between the University and the Agricultural Research Council, constituted a Unit of the Agricultural Research Council, with the title of the Agricultural Research Council Unit of Plant Nutrition (Micro-Nutrients). Under the new arrangement the staff of the Scientific Officer and Experimental Officer grades became officers of the Council. The Station Director was appointed Honorary Director of the Unit and Dr. E. J. Hewitt was seconded from the Station staff to work in the Unit. The technical assistants remain in the employment of the University. The changes are shown in the Staff List. Under the agreement, the University continues to accept responsibility for the administration of the maintenance grants made to the Unit and has also agreed to accord academic status to members of the scientific staff of the Unit in approved cases. The new arrangement has established the work of the group on a more permanent basis than hitherto and should allow the research programme to be planned in a more satisfactory manner.

A second event of major importance was the visit of an A.R.C. Visiting Group to the Station from May 5th–7th to inspect the work in progress and to agree with the Director the general programme for the five-year period 1952–1957. The group comprised Professors C. S. Harland (Chairman), T. A. Bennet-Clark, W. Brown and H. A. Krebs. Dr. E. E. Cheesman and Mr. G. G. Samuel of the Council's staff accompanied the group, and Sir William Slater, Secretary to the Council, was present on the third day. During the visit the group was able to agree the five-year programme with the Director, and subsequently the Council has furnished a report of the visit.

Two other visits of importance by officers of the Ministry of Agriculture call for mention. On 27th February Mr. R. A. Hughes visited the Station to settle outstanding points of finance between the Ministry and the University, and agreement was reached regarding the basis of future grants and various details of administrative procedure. The second visit was made by a small party consisting of Mr. C. H. M. Wilcox, Mr. A. C. Sparks and Mr. A. B. Bartlett, who were accompanied by Mr. L. Petch from the Treasury. Their object was to familiarize themselves with the Station and its work to help in dealing with Station business in the Ministry. The visit was very useful both to ourselves and to the visitors, whom it was a pleasure to welcome to the Institute.

Sir John Simonsen, Chairman of the A.R.C. Conference on Cider and Fruit Juices, repeated his visit of 1951 to discuss with Dr. Pollard and his staff the research in progress in the Cider Section. The visit was much appreciated by all concerned, and we are grateful to Sir John for his continued interest and help.

On March 27th and 28th a Symposium, organised jointly by the Director and Dr. E. C. Bate-Smith, Director of the Low Temperature Research Station of the D.S.I.R., Cambridge, on the subject of " Tannins and Related Compounds in Plants and Food," was held at the Station. The members comprised workers on these subjects from various research centres and from the University Department of Chemistry, including Professor Wilson Baker, who contributed the introductory paper. The subjects discussed were of particular interest to our Cider and Fruit Juices Section. The symposium was an outstanding success and it is hoped to organise others from time to time on a similar basis with the D.S.I.R. staff.

During the period 30th June–12th July a special course for overseas students organised by The British Council, on the subject " Mineral Disorders in Plants with Special Reference to Trace Elements," was given by the members of the A.R.C. Plant Nutrition Unit, the Director acting as Honorary Director of the course and contributing some of the lectures. Seventeen students, all of graduate status, from nine countries, attended. Administrative matters were in the hands of Mr. J. C. Gayford of the Council's Bristol office. During the period the members of the course visited the Royal Show at Newton Abbot and spent one day on the Cotswolds when they visited the Royal Agricultural College at Cirencester.

A further extension of the Unit's activities occurred during the year, arising from the formation by the A.R.C. of a group to consider problems of animal health in relation to mineral deficiencies and excesses in crops. It was suggested by the group that experimental plots should be established to study the copper status of crops on farms where " swayback " in lambs and hypocuprosis of cattle were known to occur. In collaboration with the veterinary centres at Weybridge and Langford, four centres were selected and indicator plots, comprising special indicator crops and fertilizer treatments, were established.

At the invitation of the Rubber Research Institute of Malaya, and through the medium of the Agricultural Research Council, the Station has undertaken to carry out a special investigation on behalf of the Rubber Research Institute to determine the substances in tar oil distillates that are effective in the control of mouldy rot disease of the tapping panel of the rubber tree.

After agreement between the Ministry of Agriculture and the Ministry of Food the Station was asked to undertake research work and to provide courses of instruction on the domestic preservation of meat in addition to the present work on fruit and vegetables. This was agreed to in principle and subsequently arrangements were completed for the work to begin early in 1953. The extension has involved a new appointment in the experimental officer grade.

It was not necessary to call a meeting of the Cider Advisory Committee in 1952, but its two sub-committees on Cider Orcharding and Cider Manufacture both met. The former, at a meeting on 15th February, discussed a proposal to establish trial orchards of perry pear varieties, and preparations to do so are now in progress. A report on the centres for which final arrangements had been made for the trials of bush and standard cider varieties was also received. The centres additional to those recorded as planted in the 1951 Report are as follows :—

Bush Plantations.

Devon : Mr. E. W. Pinks, Ottery St. Mary.
Gloucester : Mr. J. T. Fortescue, Ebrington Manor Fruit Farm, Campden.
Somerset : Mr. W. E. L. Allen, West Bradley House, Glastonbury.
Worcester : Mr. C. P. Norbury, Sherridge, Nr. Malvern.

Standards.

Devon : County Farm Institute, Bicton, East Budleigh.
Seale Hayne Agricultural College, Newton Abbot.
Hereford : Mr. R. Morgan Jones, Bowling Green Farm, Clehonger.
County Farm Institute, Nieuport.
Somerset : Mr. R. J. Sheppy, Three Bridges, Taunton.
Mr. J. Barber, Manor Farm, Ditcheat, Shepton Mallet.

The Cider Manufacture Committee met on November 4th to discuss the research programme of the Cider Section for 1953.

The panel established for the training of cider judges met on February 14th and November 4th, when tasting tests were carried out under the guidance of Professor Barker.

The Management Committee of the Institute at its meeting on 15th July, recommended the award of the Memorial Studentship in cider-making to Mr. J. C. Blore, Fladbury, Worcestershire, the recommendation being later endorsed at the annual meeting of members.

Twelve members of the scientific staff visited Fernhurst Research Station on 26th and 27th June as the guests of Messrs. Plant Protection. The outing was much appreciated and thanks are due to the Company for the opportunity of inspecting the work at Fernhurst and for their generous hospitality.

The XIIIth International Horticultural Congress, which was held in London in September under the auspices of the Royal Horticultural Society, was attended by a number of the scientific staff. The Director gave one of the Congress lectures, and papers were also contributed by Dr. Hewitt, Mr. Hobbis and Dr. Luckwill.

After the Congress, Dr. F. W. Went, Director of the Earhart Plant Physiology Laboratories, California Institute of Technology, Pasadena, U.S.A., visited the Station and gave a lecture, illustrated by a film and colour slides, on the work of this notable centre of research. Members of the University staff and the local N.A.A.S. were invited, and the event was greatly appreciated by all. Our thanks are due to Dr. Went for coming to Long Ashton and giving this stimulating lecture, and to the Agricultural Research Council for providing a special grant to meet expenses incurred.

On September 30th, 1952, the Chipping Campden Fruit and Vegetable Research Station severed its connexion with the University, which had been responsible for its management since 1923. In future, the Station will function as the Research Station of the Fruit and Vegetable Canning and Quick Freezing Research Association, and will be financed by grants from the Department of Scientific and Industrial Research, and contributions from the member firms of the Association. In making the change it was felt that a stage had been reached when the further development of the Station could best be promoted under the direct management of the appropriate Trade Association, and within the general scheme of the D.S.I.R. for such organizations. The break between the University and the Station was made not without feelings of regret, for the University had nurtured the Station from very small beginnings, through many difficult times, to its present stature and reputation, and relationships between the University and the industries connected with the Station had always been most cordial.

It was recognised, however, by all parties concerned that in the interests of the Station the change was necessary and to this the University readily agreed. The best wishes of the University and of Long Ashton Station go with Campden in its new venture, and they look forward to its continued growth and success. It is hoped that the University will maintain informal contacts with the work of Campden through Long Ashton and the Department of Chemistry.

It is pleasing to record, in the closing years of the long service of Mr. Spinks, the Station plant breeder, that a number of his seedlings are now attracting special notice. During the year, his new pear, Bristol Cross, which is now grown commercially, and the plum Severn Cross gained Awards of Merit from the Royal Horticultural Society. It is hoped that other promising varieties now under trial will be similarly recognised.

In view of Mr. Spinks' approaching retirement it is of interest to note that the Agricultural Research Council has recently considered a national policy for fruit breeding, and accepted the future programme for the Station.

It is with deep regret that we record the passing of Professor F. T. Brooks, F.R.S., who from 1937 to 1946 represented Cambridge University on the Governing Body of the National Fruit and Cider Institute. Being a Somerset man, he was at one period a frequent visitor to the Station and was especially interested in the mycological section.

Following the resignation of Mr. R. G. W. Bush and the changes at Campden, Mr. A. Woodall has been appointed as the Ministry of Agriculture's representative on the Agricultural Committee. Mr. C. E. Hudson, Senior Education and Advisory Officer, Ministry of Agriculture and Fisheries, has also been co-opted to serve on this committee to provide a direct link with the Ministry's Scientific Officers.

Staff.

There were a number of changes in the scientific staff during the year, the details of which are recorded below.

Appointments.

Mr. R. R. Williams was appointed as Cider Pomologist in succession to Mr. J. D. Cuthbertson from 10th January, 1952. Mr. Williams is a graduate in Botany of the University, and after taking his degree he obtained the University Diploma in Horticulture. During the war he served in the R.A.F. as a member of an aircrew, and has also had experience in managing the family small-holding.

Mr. G. V. Coles was appointed to the temporary post of Organic Chemist on 4th February, 1952, to undertake the investigations under the special grant from the Rubber Research Institute of Malaya. Mr. Coles, who is a graduate of the University of Wales, has had extensive experience in petroleum chemistry in Persia as a chemist of the Anglo-Iranian Oil Company.

Mr. C. P. Lloyd-Jones was appointed to a new post in the Physical Chemistry Section on 11th August, 1952, to extend the work on radio-active isotopes in collaboration with Dr. Thomas. Mr. Lloyd-Jones is a graduate in chemistry of the University, and during his period of National Service was engaged in a radar section.

Mr. A. T. K. Corke was appointed as Assistant Mycologist on 1st September, 1952, to succeed Mr. S. H. Crowdy. He is a graduate of Edinburgh University, where he held the post of Assistant Lecturer in Botany. From 1940 to 1946 he served in the Inns of Court Regiment, being abroad in France in 1944 and in India during 1945 and 1946.

Mr. M. Greenwood was appointed as Head of the Technical Section on 20th October, 1952, to succeed Mr. H. S. D. Swan. Mr. Greenwood, who is a graduate in chemistry of Manchester University, has had long experience in the tropics, particularly in West Africa, as a chemist in the service of the Colonial Office. He was created O.B.E. in 1950 and retired from the Colonial Office service in 1952. His previous experience should be of great value in his new post.

Dr. C. C. McCready was appointed as Plant Physiologist in the A.R.C. Unit of Plant Nutrition in succession to Dr. E. W. Bolle-Jones on 17th November, 1952. Dr. McCready is a graduate of Oxford University where he has been engaged in research since graduation.

Mr. K. G. Stott, Willow Officer, returned from his period of National Service on 16th April, 1952. During his absence he served as an educational officer in the Royal Air Force.

Appointments to assistant staff were as follows :—

Mr. D. F. Day, Assistant Experimental Officer, as Greenhouse Officer, A.R.C. Unit of Plant Nutrition, from 21st January, 1952. Mr. Day is a graduate in horticulture of Reading University ; he was a student at the R.H.S. gardens, Wisley, and has had commercial experience. He served in the Royal Corps of Signals in the Middle East during the war.

Mr. J. F. Harris, Assistant Experimental Officer, as Analyst, Insecticides and Fungicides, from 1st September, 1952. Mr. Harris received his training in the City Analyst's Department, Bristol.

Mr. D. W. Burd, Assistant Experimental Officer, Pomology, from 1st October, 1952. Mr. Burd served with the Royal Engineers in Egypt and Palestine from 1945 to 1948, and after two years' previous exployment at Long Ashton between 1948 and 1950 he took a Diploma Course in Horticulture at the Essex Institute of Agriculture, Writtle, passing the N.D.H. Intermediate Examination in July, 1952.

Mr. P. S. Weaver, Assistant (Sc.) Pomology, from 1st February, 1952.

Miss S. J. Locke, Assistant (Sc.), Cider Chemistry, from 31st March, 1952.

Miss P. M. Jackson, Assistant (Sc.), Organic Chemistry, Rubber Research Institute Grant, from 21st April, 1952.

Miss B. Hood, Assistant (Sc.), Chemistry, Soils and Plant Nutrition, from 8th September, 1952.

Mr. R. E. Monro, Assistant (Sc.), Temporary Appointment, Chemistry, Soils and Plant Nutrition, from 6th October, 1952.

The following promotions of members of assistant staff were made :—

Mr. A. J. Sims, promoted from Assistant (Scientific), Physical Chemistry, to Assistant Experimental Officer from 1st October, 1952.

Miss J. H. Baker promoted from Assistant (Scientific) Microbiology to Assistant Experimental Officer from 1st October, 1952.

Mr. R. M. Jarrett promoted to Experimental Officer from 1st October, 1952.

Resignations.

There were three resignations from the Scientific Staff and one temporary worker completed her period of service.

Mr. S. H. Crowdy resigned from 31st March, 1952, to take up an appointment in Imperial Chemical Industries.

Mr. H. S. D. Swan resigned from 31st August, 1952, to take up a post at the Pulp and Paper Research Institute, Montreal, Canada.

Dr. E. W. Bolle-Jones (A.R.C. Unit of Plant Nutrition) resigned from 6th July, 1952, for an appointment in the Rubber Research Institute, Malaya.

Miss H. M. Ferres completed her period of temporary employment in the Pomology Section on 31st January, 1952.

Resignations of Assistant Staff were as follows :—

Mrs. B. A. Baker, Assistant (Sc.), resigned from 31st March, 1952.

Mr. N. A. Wright, Assistant (Sc.), resigned from 16th February, 1952.

Administrative Staff.

Appointments.

Miss S. R. Littlewood, from 31st March, 1952.

Miss B. Chapman, Clerical Officer, Temporary Appointment from 3rd November, 1952, to replace T. J. Ford, called up for National Service.

Resignations.

Miss M. A. Febrey, Shorthand-Typist, from 9th February, 1952.

Mrs. J. I. Packer, Telephonist, from 31st March, 1952.

National Service.

Mr. T. J. Ford, Clerical Officer, left for his National Service training on 20th October, 1952.

Temporary Workers.

The following have been accommodated in the Institute laboratories during the year :—

Dr. H. Gleen, A.R.C., who continued his work on soil metabolism.

Miss P. Miles, N.A.A.S., continued her work on bulb nutrition.

Dr. S. C. Agarwala continued his investigations under a temporary grant from the A.R.C. on completing his period as a graduate student, from 6th May to 31st October, 1952.

Visits Abroad.

Dr. H. G. H. Kearns visited Jamaica on behalf of the Colonial Office for the period 1st September to 2nd October, to advise on problems connected with the spraying of crops in the island.

He also visited Malaya at the request of the Colonial Office for the period 4th–23rd December, 1952.

Dr. D. J. D. Nicholas, A.R.C. Unit of Plant Nutrition, was granted leave of absence for one year from 15th September, 1952, to hold the E. J. Johnson Post-Doctoral Fellowship at Johns Hopkins University, Baltimore, U.S.A.

The Director visited Dublin on March 19th to lecture to the Institute of Chemistry of Ireland.

The Director, with Drs. C. Bould, E. J. Hewitt, D. J. D. Nicholas and W. Plant, attended the meetings of Commissions II and IV of the International Society of Soil Science in Dublin, 21st–31st July, 1952.

Dr. L. C. Luckwill, Mr. E. W. Hobbis and Miss B. A. Rake attended the Horticultural Education Association Conference in Eire and Northern Ireland, 1st–4th September, 1952.

Dr. J. T. Martin and Messrs. R. W. Marsh and N. G. Morgan attended the 3rd Crop Protection Congress in Paris, 14th–20th September, 1952.

Degrees, etc.

The following were awarded the Degree of Ph.D. in the University :—

Mr. E. J. Skerrett for a thesis entitled "Chemical Studies in the D.D.T. Series."

Mr. E. W. Bolle-Jones, for a thesis entitled "The Inter-Relationships of Iron and Potassium in the Potato Plant."

Dr. S. C. Agarwala, for a thesis entitled "A Study of the Molybdenum Nutrition in Cauliflower."

Dr. E. J. Hewitt published a book entitled "Sand and Water Culture Methods used in the Study of Plant Nutrition" as Technical Communication No. 22 of the Bureau of Horticultural and Plantation Crops, East Malling, Kent.

Buildings and Land.

In the 1951 Report reference was made to the proposals for further buildings that had been submitted to the A.R.C. and the Ministry of Agriculture, and to the fact that the Ministry had authorised the preparation of plans for the items in Priority A of the Scheme, comprising a Store for the Biology Laboratory to serve as temporary laboratories, an extension to the preparation room and the provision of extra lavatories in the main laboratory building, and a farm store to replace a dilapidated shed standing on the proposed site of the new laboratories. During the year all these items were proceeded with, excepting the lavatories, for which the work will be undertaken in the spring of 1953. The extension to the preparation room is completed and is now in use, the farm buildings are in course of erection, and the temporary laboratories should be completed for use in the summer of 1953. Whilst these items are all welcome additions to our accommodation, the serious congestion at present interfering with the progress of certain lines of work will continue until the major items of the building programme are complete.

As the result of an agreement reached with the Ministry of Agriculture, it was possible to proceed with some alterations and renovations in connexion with the cider factory. The main items were the concreting of the factory yard, the adaptation of a portion of the factory building as a fermentation room (including the installation of special temperature control fitments), the treatment of walls and ceilings of various sections of the building with anti-mould preparations, and the re-wiring and overhaul of electric equipment. These various improvements will enable the building to be used for many types of experiment hitherto impossible because of contamination from unwanted micro-organisms that could not be kept under satisfactory control.

An extensive programme of painting and repairs was also undertaken on the greenhouses ; the large E.M.B. greenhouse was thoroughly overhauled, the work including the stripping and reglazing with plastic putty of a large section of the roof, with the object of rendering the house more suitable for experiments on micro-nutrients. A large section of the interior of the Director's House was painted and redecorated.

On the farm some further clearing of the older experimental areas was undertaken and a proportion of the previously cleared areas was replanted. The details are as follows :—

Clearing and thinning.

Plots 9 *and* 10. The Damsons along the side of Plot 9 and various apple trees on Plot 10 have been removed to make way for a planting of new varieties of fruit raised at Long Ashton.

Plot 22. One-third of the three-quarter standard Bramley and Worcester trees have been grubbed on the lower end to make way for new blackcurrant and gooseberry trials.

Planting.

Plot 5. Further series of seedling apples, mainly of cider varieties, have been planted for preliminary observations.

Plot 6. Further additions have been made to the Cider Scion Nursery, which now contains 53 varieties, including each on the recommended list.

Plots 9 *and* 10. Eighteen trees, comprising new varieties, some already in commerce, of apples, pears and plums raised by Mr. Spinks have been planted as a long-term record of the new varieties originating at Long Ashton.

Plots 13 *and* 16. The scheme to plant dwarf pyramids on this area will be completed in 1953-54. So far 1,562 trees have been planted, out of an ultimate total of about 5,000.

Plot 21. 2·4 acres of the upper part of this plot has been planted to 325 " high " and " low " worked plum trees on Myrobalan " B " rootstock, of the varieties, Victoria, Czar, Warwickshire Drooper and Giant Prune. These trees are concerned with work on the incidence of and resistance to Bacterial Canker.

Research Activities.

Following the establishment of the Agricultural Research Council Unit of Plant Nutrition on 1st April, 1952, it has been decided to report the results of the work of the Unit separately from those of the other sections of the Station in this account.

Fruit Tree Nutrition and Soils.

The results of cover crop treatments on cider apple trees have been summarised for the period 1947–1951. The main effects on nutrient status are the lower nitrogen and higher potassium and phosphorus contents of the leaves of trees in permanent grass. Effects on magnesium status were not significant, but magnesium content was higher for trees on Crab than on Malling I rootstock. A new experiment on bush apples, combining N.P.K. manurial combinations and cultural systems, was begun.

In experiments on the use of foliage sprays on apple trees there was no residual nitrogen effect from urea spraying in the season following the treatment. Urea sprays checked the seasonal decline in the nitrogen content of the leaves during the season of treatment. Petal fall application was more effective than at pink bud.

In preliminary experiments with versenates (iron complexes of ethylene diamine tetra-acetic acid) the uptake of iron by rye seedlings was increased.

The nitrogen content of blackcurrant leaves, previously associated with optimum yields (2·9% of dry matter), has not been achieved in later seasons by continuing previous optimum fertilizer treatment ; the nitrogen levels from the various treatments given show a progressive decrease as the experiment proceeds. It remains to be determined whether or not this is a natural consequence of age of bush.

The effects of omitting N.P. and K. respectively in the experiment on Auchincruive Climax Strawberry, previously reported, were again shown in 1952—the third year after planting. The result that phosphate omission has not decreased either growth or yield after its continuous omission since 1923 is of particular interest.

In pot experiments on the effects of the soil conditioner ' Krilium,' using a fine sandy silt loam soil, the addition of ' Krilium ' at rates between 0·05 and 0·2% of the air-dried soil did not adversely affect the uptake of phosphorus and potassium by rye seedlings. A field experiment on strawberries on a similar soil has also been started.

Pomology and Plant Breeding.

Further progress was made in the application of chromatographic methods to problems of plant hormones. Thiourea in ether has been used successfully to extract auxins from apple tissues rich in oxidising enzymes. Extraction of blackcurrant fruits at weekly intervals from fertilization to maturity showed at least three different auxins and one inhibitor to be concerned in fruit development. In apple fruits four auxins and one inhibitor were found.

In the leaves of winter cauliflower the presence of both indolyl acetic acid and indolyl acetonitrile was demonstrated. No difference was found between the auxin content of healthy plants and others deficient in molybdenum. Indolyl acetic acid was found in asparagus berries, gooseberries, young maize cobs, leguminous root nodules and cabbage, but not in apple or pear.

In continuation of work on the activity of naphthoxy compounds, the activity of fifty of these has been measured by the tomato parthenocarpy test. The fruit thinning properties of different concentrations of naphthalene acetic acid (NAA) have been determined, using the apple varieties Millers Seedling, Edward VII and Crawley Beauty.

Spraying with NAA (20 and 50 p.p.m.) at full bloom on pears increased the yield of Conference, but decreased the yields of Beurre Bedford, Laxton's Superb and Williams. 2-naphthoxy-propionic acid at these concentrations always decreased the yield. 2:4:5-trichlorophenoxy-propionic acid at concentrations of 10–100 p.p.m., applied 8, 12 or 15 weeks after petal fall, hastened maturity of Worcester Pearmain apples. Using 50 p.p.m. at 15 weeks after petal fall, fully coloured fruits of normal size and quality were obtained two weeks earlier than on unsprayed controls.

The use of apomictic seedlings of *Malus toringoides*, *M. sikkimensis* and *M. theifera* as rootstocks for the apple varieties Lord Lambourne, Worcester Pearmain and Cox Orange Pippin is under investigation.

Tests for incompatibility of new varieties of plums suggest that Frome Cross is incompatible with Marianna and Common Plum. Thames Cross also failed on Marianna. All varieties have been successful so far on Brompton. The effect of stock foliage on incompatible graft combinations of Peach, Myrobalan B and Brompton is being examined.

The virus causing rubbery wood of apples was found to be widely distributed in commercial stocks of M.I rootstock, whereas most samples of M.II rootstock appeared free of the virus. Of 44 different varieties tested for resistance against the vein banding strain of apple mosaic, 9 appeared tolerant, 11 partially tolerant and 24 susceptible.

Observations were continued on the cropping of established bush cider apple plots and four new trial plots were planted. A survey of perry orchards was made in N.W. Gloucestershire and about 100 fruit samples collected for vintage trials.

Pollination tests with cider apple varieties were continued. Of 75 varieties 12 gave notably low results for germination, suggesting triploid characters. Leaf and flower characters as aids to the identification of cider varieties were further studied. The fruit characters of 80 varieties of perry pears have been described.

A new method of treating plum trees in the nursery, which avoids winter pruning, has been evolved. The method is described later in this volume.

Preliminary trials on blueberry culture in West Somerset have been published.

Among the seedlings raised at the Station, one variety of pear, Bristol Cross, and one plum, Severn Cross, have been given Awards of Merit by the Royal Horticultural Society. A variety of apple, Cheddar Cross (Allington Pippin crossed with Star of Devon) is to be distributed to sub-centres for further trial under the National Fruit Trials scheme. Another apple seedling, W.B.B.54, has also been included in the National Trials. Other breeding work is in progress for the production of new cider varieties, early dessert plums and blackcurrants of high ascorbic acid content.

Pests and Diseases and their Control.

The interactions of fungicides with fungus enzymes are being explored in joint work by the Chemistry and Plant Pathology Sections. It has been found that a non-damaging derivative of the phytotoxic compound dichloronaphthaquinone can apparently be converted to an active fungicide by the enzymes produced by germinating spores. Investigations on fungus catalase systems have shown that sulphur inhibits catalase almost completely, while the effect of copper varies with the fungus species employed.

Tar preparations used against the mouldy-rot fungus of rubber tree tapping panels have been fractionated and bio-assayed : emulsions of the most promising fractions were sent to Malay for field trial.

The use of radioactive tracers in joint work by the Chemistry and Entomology Sections has permitted studies of the absorption, translocation and breakdown of the organophosphorus systemic insecticide, schradan, in crop plants. The possibility of schradan contamination of nectar, and consequently of honey, has been demonstrated.

Field experiments by the Plant Pathology Section have shown prolonged suppression of sporulation on apple cankers following spraying with phenyl mercury chloride or sodium pentachlorphenate. Against apple scab, the value of the non-phytocidal phthalimide spray, S.R.406, has been confirmed. An increase in apple mildew attack was recorded following three years' treatment with a glyoxalidine spray.

By the use of an apparatus constructed to give reproducible spray deposits and to simulate rain washing the tenacities of copper oxide films on a variety of surfaces have been evaluated. Droplets of known volume and pattern of distribution have been used on bean leaves to assess the range of toxic effect against spores of *Botrytis fabae*. Copper deposits from such droplets exert a fungicidal action when linked to the spores by a water bridge.

Trials on the persistence of spray residues on gooseberries and raspberries have proved that there is little risk of excessive D.D.T. residues provided that the fruits are sprayed at an early stage of development.

Field applications against red spider mite on apples have shown that, in hydraulic spraying, control is increased by using spray brooms adapted for tree shape. In airflow applications against red spider it has been found necessary to have adequate volume and high velocity of air to lift the leaves to expose their lower surfaces. Low-volume spraying with lime sulphur has given adequate scab control, but a lime sulphur concentrate of 4% strength caused serious fruit drop on Worcester Pearmain apples.

Automatic sprayers for dwarf pyramid apples and for blackcurrants have been improved, and equipment was designed for N.A.A.S. herbicide applications, for the Scottish Horticultural Research Station and for the application of malaricides in the tropics. A visit was made to Jamaica from 1st September to 2nd October, 1952, to report on the materials, equipment and methods used in spraying bananas for the control of leaf spot disease. A report on this visit has been made to the Colonial Office.

Cider and Fruit Juices.

Cider and apple juice. In further experiments on the effect of stage of maturity on the vintage quality of cider, highest vintage quality and maximum specific gravity were found at the stage of maturity normally used for cider-making. Deterioration in cider quality became serious only at a very late stage.

Treatment with cation exchange resins combined with paper chromatography has been successfully used in the separation of nitrogenous fractions of apple juices and ciders. In juices asparagine was the main amino acid present : the ciders contained only minute traces of amino acids.

The contents of metals that may possibly occur as contaminants in apples and their products, and the effects of lead and arsenic sprays applied to apple trees on the contents of these elements have been determined. With regard to the latter point, it was shown, in considering the concentrations of the elements in the fresh fruit, juice, cider, pomace and pectin, that lead only occurred in quantity in the fresh fruit and arsenic in the fresh fruit, juice and pectin.

Washing the sprayed fruit with water was ineffective in the removal of both elements ; washing with 1% HCl decreased the lead by about 50% and the arsenic by 30% in the fruits.

In an attempt to relate the changes in apple juice polyphenols with deposit formation during storage, no direct relation with polyphenol content or with polyphenolase activity was found. SO_2 used in small amounts was beneficial as an oxidation inhibitor.

The non-volatile organic acids of apple juices and ciders have been separated from the other constituents by ion exchange treatment and paper partition chromatography, and methods devised for their subsequent quantitative estimation. The methods have been applied to study the changes that occur during the course of fermentation either by naturally occurring or selected yeasts, and to determine the effects of prefermentation treatments. Juice acidity was found to be mainly due to malic acid with smaller quantities of quinic and phosphoric acids. In the early stages of fermentation there was an increase of acidity due largely to succinic acid. Later, in the dry ciders, the acidity fell, due to the replacement of malic acid by lactic acid, except where pre-treatments had inhibited malo-lactic fermentation.

Juice treatments necessary for the restriction of the natural flora of apple juice were found to be important in determining the course of subsequent fermentation when selected yeasts are added. In studies of yeast dominance, it was shown that both morphological and biochemical tests are necessary to assess the degree and nature of the dominance achieved. Deficiencies of nitrogen and aneurin have been shown to be limiting factors to yeast growth and rate of fermentation.

The function of lactic acid bacteria in cider-making has been further studied and some of the organisms producing malo-lactic fermentations have been used successfully for lowering the acidity of sharp dry ciders.

An organism capable of producing the disorder of cider known as oiliness or ropiness has been cultured in ciders produced by different treatments from a single juice, and it has been shown that the organism can grow in both juice and ciders without the production of ropiness. An apparently normal cider may, therefore, be a source of infection for a susceptible cider following blending.

Providing the initial acidity of ciders to be used in the manufacture of cider vinegar is at least 1%, the acetic bacteria from either malt vinegar or cider may be used, but the flavour of the vinegars made with bacteria from the former source appears to be superior.

Flash pasteurisation of apple juice appears to offer a satisfactory method of treatment for the bulk storage of the juice. It has been shown that the method ensures the retention of freshness of flavour and can offset the development of cooked flavours in subsequent tank pasteurisation. The sterilisation of the containers, however, may present difficulties in using the method, but the use of an atmosphere of carbon dioxide may assist in this problem. Flash pasteurisation also appears suitable for the bulk treatment of cider, though the treatment of juices for cider-making prior to fermentation may present difficulties.

Soft fruits and their products. A further season's work on the effects of manurial treatments on the quality and composition of strawberries has confirmed the importance of an adequate level of potassium in ensuring high quality. In addition to poorer flavour, deficiency of potassium was associated with lower acidity, ascorbic acid and total sugars content. Comparison of blackcurrant juice concentrates prepared by a laboratory climbing-film evaporator at 40–50° C., by a low temperature commercial evaporator, and by freeze concentration, emphasised the importance of retaining the volatile ester fractions. Ascorbic acid retention was good in the concentrates produced by all three methods.

Domestic Preservation of Fruit and Vegetables.

Further experiments on heat penetration in bottled and canned fruits have led to the recommendation of three new methods, a quick-heating method in water, a pressure method utilising steam, and an oven method. Factors that have been considered when recommending the processing times are : the pH value of the fruit, which has an effect on the kinds of micro-organisms likely to cause spoilage, the temperature required to inactivate the oxidase enzymes of light-coloured fruits, the proportions of fruit to liquid in the jars and the quantity being processed at once.

The temperatures required to inactivate the oxidase enzymes of pears and plums have shown the necessity of high temperatures for some varieties of plums, which may result in an over-cooked condition of the fruits. The amounts of gas and electricity used when preserving different amounts of fruit by these methods have been recorded.

Extensive trials have been made to compare the quality of varieties of fruits and vegetables preserved by freezing and by bottling or canning. It has again been found that the varieties most suitable for freezing are often not the best for bottling or canning, and that seasonal variations necessitate trials over several years before reliable results can be obtained.

Willows.

Preliminary work on the botanical characters of varieties within single species has shown that many vary greatly on different sites and that the identification of varieties may often be difficult for this reason.

Agricultural Research Council Unit of Plant Nutrition (Micro-Nutrients).

Molybdenum Nutrition.

Comparison of growth and visual symptoms in beans and peas grown in sand cultures from seed saved from previous deficiency treatments and from fresh commercial seed, revealed large differences in susceptibility due to seed history. Commercial seed provided sufficient reserves to mask the visual symptoms and yield effects of molybdenum deficiency that appeared in plants grown from depleted seed. Seed history also affected yield performance in complete nutrient cultures but to a much less extent.

Severe visual symptoms were produced in leaves of potato plants grown without molybdenum from tubers saved from previous experiments. There was a progressive reduction in molybdenum status of deficient plants subjected to this treatment over a period of three seasons.

Results obtained in 1951 with cauliflower on the relation of molybdenum requirements to nitrogen source were confirmed and additional evidence of a molybdenum requirement with nitrite and glutamic acid were obtained. Whiptail effects and growth reductions were caused by molybdenum deficiency irrespective of a wide variety of nitrogen sources.

Depression of chlorophyll occurred with the use of nitrate nitrogen. Molybdenum did not affect chlorophyll when ammonium compounds were given in the absence of nitrate. The nitrite effect was intermediate. Wilting and withering symptoms were always associated with nitrate treatments in the absence of molybdenum. Total sugars, reducing sugars and sucrose were depressed by molybdenum deficiency irrespective of nitrogen sources except urea. The ratio ascorbic acid/dehydroascorbic acid was slightly but usually significantly decreased by deficiency or excess of molybdenum.

Protein and amino nitrogen were reduced by molybdenum deficiency in plants given nitrate or nitrite when calculated either as total concentration present or as a proportion of total nitrogen. Total concentration of protein and amino nitrogen were not reduced when ammonium compounds or urea were given, but total nitrogen was increased in these treatments. There were sometimes reductions in protein and amino nitrogen in these treatments when results were expressed as a proportion of total nitrogen in the plants. The concentrations of protein and amino nitrogen in molybdenum deficient plants given ammonium compounds or urea were usually greater than those in plants given nitrate and molybdenum, and these nitrogen fractions were not considered as direct limiting factors in the growth of deficient plants. A role of molybdenum additional to that in nitrate reduction is inferred.

In these experiments arginine was the major free amino acid in the leaves of the molybdenum deficient cauliflowers when ammonia or urea was the source of nitrogen.

The R.Q. of cauliflower leaf discs was increased to about 1·1 in molybdenum deficient plants given nitrate. With all other nitrogen sources or in the presence of molybdenum the value was closer to 1·0. Molybdenum injection of deficient plants given nitrate caused a further rise of R.Q. to 1·3 and then a fall to 1·0. This behaviour was paralleled by a rapid nitrate reduction in the tissues.

In a field experiment on a molybdenum deficient soil, where cauliflower has been grown for three successive seasons to test long period effects of lime, gypsum and sodium molybdate applied to the soil and the treatment of seedlings with molybdate previous to planting on the plots, the seedling treatment was most effective in controlling Whiptail. Gypsum increased the trouble.

Iron Nutrition.

In factorial experiments with potato in sand culture to examine the inter-relationships of iron, potassium and phosphorus, increased potassium increased total iron concentration and decreased phosphorus. Potassium increased the efficiency of chlorophyll production per unit weight of iron in iron deficient plants. Chlorophyll content appeared to be positively correlated with increased amounts of iron extracted from old leaves by 0·1 N.HCl and ether, and with increases in the ratios of soluble Fe/soluble P and total Fe/total P in the plants, and was negatively correlated with the ratio soluble P/total P. Chlorotic leaves had a higher K/Ca ratio than green ones in potassium deficient plants, irrespective of whether or not calcium carbonate was mixed with the sand. Extra iron caused a decrease in the ratio of potassium in young to old leaves.

There were interactions between potassium and phosphorus status of the plants in regard to the proportions of iron, potassium or phosphorus absorbed from the total amounts presented in the nutrient solutions.

The total content of iron, potassium and phosphorus per plant, and the relative distribution of the elements in different plant parts were profoundly affected by changes in their relative status in the plant and by numerous interactions.

In pot experiments, using tomato in soil, iron deficiency effects were produced by cobalt and nickel, but not by cadmium, all being added at 7·5 and 15 milli-equivalents per pot (1·6 kg. soil). Iron deficiency was also produced in oat by copper and cobalt.

Copper and Zinc Nutrition.

Effects of seed reserves on the production of copper and zinc deficiencies in large seeded legumes were investigated. Marked differential reduction in growth and severity of visual symptoms were obtained for copper and zinc deficient plants grown from depleted seed. Effects on yields were greater for copper than for zinc deficiency, but plants from both depleted and fresh commercial seed showed visual symptoms for each deficiency whereas only plants grown from depleted seed showed molybdenum deficiency symptoms.

Essentiality of Cobalt, Nickel and Gallium for Plants.

Using highly refined purification methods in sand cultures, no evidence of essentiality of these elements for tomato was obtained.

Soil Conditioners.

In a pot experiment the addition of ‘ Krilium ’ to a clay soil deficient in manganese increased the manganese uptake of oat only in the presence of added manganese.

Phosphorus-32. P^{32} was used successfully to examine the effect of nickel on the uptake of phosphorus by tomato from a phosphate deficient soil.

Mineral Nutrition of Micro-Organisms.

Aspergillus niger has been found unsuitable for determining available iron in soils as the fungus obtains adequate iron for full growth from so-called iron deficient soils.

Progress has been made in developing methods for the determination of the mineral nutrient requirements of some heterotrophic and autotrophic bacteria. Difficulties occur in purifying solutions as the usual methods may remove essential organic nutrients.

The function of trace metals in the metabolism of micro-organisms is being investigated, in particular the production of B vitamins by *Aspergillus niger* given graded amounts of Fe, Cu, Zn, Mn and Mo. Vitamin B.12 is produced by *Aspergillus niger* in the presence of cobalt. Using Warburg manometric methods preliminary experiments have indicated differential activity of catalase and peroxidase in homogenates of fungi, using mainly *Aspergillus niger*, in relation to the supply of specific micro-nutrient elements.

Organic Constituents of Plants.

Considerable progress has been made in the characterisation of the substances which make up the tannins of apple and pear juice. In addition to chlorogenic acid which, as previously reported, occurs in both juices, epicatechin is an important constituent of cider apples, while substances resembling leuco-anthocyanins of various types have been found in quantity in both cider apples and perry pears.

It has been confirmed that arginine becomes the major free amino-acid of cauliflower leaf when plants are grown in sand culture with ammonium or urea sources of nitrogen under conditions of molybdenum deficiency.

Soil Acidity.

The visual symptoms shown by a number of brassica crops—cabbage, Brussels sprouts, kale, rape and cauliflower—were determined on an acid soil pH 4·0–4·5, under the four treatments, nil, ground limestone, ground Dolomite and gypsum. The main visual effects of acidity were manganese toxicity, aluminium toxicity and molybdenum deficiency, the various crops showing these to markedly different extents. On the treated plots magnesium deficiency was the main symptom and was particularly prevalent on the gypsum plots.

Indicator Plots in relation to Copper Deficiency Conditions in Sheep and Cattle.

Indicator crop plots, including factorial fertilizer combinations of N.P.K.Cu were laid down at four centres at which copper deficiency occurs. Growth responses in the crops were generally marked for phosphorus and sometimes for nitrogen but were usually small for potassium. There were no responses to copper treatment in any fertilizer combination.

Other Activities.

Members' Day.

The Annual Field Day for Members and the Annual General Meetings of the Members and Governors of the National Fruit and Cider Institute were held on 15th July, 1952.

The main items of the programme were as follows :—

1. *Field plots.* Pear scab control ; foliage spraying with mineral nutrients—nitrogen, magnesium and manganese ; manuring of strawberries and potatoes.
2. *Pot culture exhibits.* Molybdenum requirements of cauliflower ; copper, zinc and molybdenum deficiencies ; heavy metal toxicity in sugar beet.
3. *Cider house exhibits.* Pollination problems in cider orchards ; cider apple varieties recommended for planting ; crop records of varieties from trial bush orchards ; present distribution of cider orchards ; yeasts and bacteria in cider-making ; apple juice manufacture.
4. *Laboratory exhibits.* (a) Main Laboratories—Plant hormones ; fruit-thinning sprays ; methods of hormone assay ; application of paper chromatography in the analysis of apple juices and ciders ; radio-active tracer technique for investigating fertiliser problems ; chemical and biological methods of diagnosing the mineral status of crops and soils ; methods of freezing fruit and vegetables for the home. (b) Biology Laboratories—Spray nozzle design ; insecticide and fungicide investigations.

Cider Sampling Day.

The Annual Sampling Day was held on May 1st, the morning being devoted to sampling ciders of the 1951 season. This was the third consecutive year of good cider crops and the specific gravities of the juices were on the average slightly lower than in 1950. The ciders made in 1951 were characterised by very slow rates of fermentation.

The ciders introduced in the first part of the tasting programme were from single varieties of fruit. The second section illustrated the effects of different juice treatments on subsequent fermentation with the naturally occurring yeasts. In the third group the ciders had been made from non-cider fruit—two dessert varieties and one culinary. In a year when cider fruit is in short supply it may be necessary to make some use of cull market fruit, but one of the main problems involved is the high acidity of culinary varieties such as Bramley's Seedling. The ciders in this group showed two ways of overcoming this problem. The last group consisted of cider blends of varieties grown in five different localities and illustrated the type of variation that can be expected between districts in any season.

During the afternoon Members had an opportunity of seeing exhibits in the Cider House and Laboratories and parties were conducted to view a bush cider plot thinned for automatic spraying trials, and plots showing nursery propagation and building of cider trees, systems of planting for apples and dessert varieties of plums.

Visitors.

The number of organised parties visiting the Station was forty-three, four less than in 1951. The full list is given below.

In addition to participants in courses arranged in Domestic Preservation, Mineral Disorders in Plants (British Council), Pruning and Refresher Courses (N.A.A.S. Horticultural Officers), it includes members of the Symposium on Tannins, of the Microbiological Group of the Societies of Chemical Industry and Public Analysts and of the A.R.C. Fungicides Sub-Committee.

Agricultural Research Council Fungicides Sub-Committee.
Association of Agricultural Education, Rural Domestic Economy Section.
Birmingham University, Extra-mural Horticultural Class.
Bristol Townswomen's Guild, Dovercourt Road Section.
Bristol Townswomen's Guild, Redland Branch.
Bristol Townswomen's Guild, Stoke Bishop Branch.
Bristol University Association of Alumni.
Bristol University Microbiology Students.
British Council Course on Mineral Disorders in Plants.
Cambridge University Agricultural Society.
Cheltenham Grammar School, Science Students.
Chepstow Society, Natural History Section.
City of Bath Training College.
Clifton College Young Farmers' Club.
Clifton High School for Girls, Science Students.
Cornwall County A.E.C. Beekeepers.
Devon Federation of Women's Institutes.
Diocesan Training College, Barrow Court.
Domestic Preservation Courses (2).
Dundry Produce Guild.
Ferris & Co., Bristol Staff.
Fishponds Social Club.
Gloucester Training College, Domestic Science Students.
Horticultural Trades Association, Bristol Branch.
Keynsham Young Farmer's Club.
Kingswood Secondary Boys' School.
Monmouthshire Institute of Agriculture.
N.A.A.S. Agricultural Officers Eastern Province.
N.A.A.S. Horticultural Officers' Pruning Course.
N.A.A.S. Horticultural Officers' Refresher Course.
Norwegian Student Visitors.
Portishead Young Conservatives.
Reading University Horticulture Students.
Redland Church Youth Fellowship.
Redland Training College Geography Students.
Society of Chemical Industry : Society of Public Analysts, Microchemical Group.

Somerset Farm Institute Horticultural Students.
Somerset Farm Institute Agricultural Students.
Somerset Fruit Growers' Club.
Somerset Teachers' Nature Club.
St. John's Family Circle, Fishponds.
Symposium on Tannins and Related Substances in Plants and Foods.

An unusually large number of visits was received from overseas scientists, some of whom had been attracted to Britain by the International Horticultural Congress held in London in September. Some of the visitors worked in the laboratories for short periods.

Visitors from Overseas (1952).

Australia :	Mr. P. B. Carne, C.S.I.R.O.
	Mr. C. G. Greenham, Division of Plant Industry, Canberra.
	Mr. E. G. Hall, Division of Food Preservation, Homebush, N.S.W.
	Dr. F. E. Huelin, Division of Food Preservation.
	Dr. J. T. Hutton, Waite Agricultural Research Institute, Adelaide.
	Mr. H. J. Kemp, South Australia.
	Professor Lauder, University of Brisbane.
	Mr. J. McDonald, Farm Services Ltd., Loxton, South Australia.
	Professor J. A. Prescott, Director, Waite Agricultural Research Institute, Adelaide.
	Mr. F. C. Pyke, Victoria.
	Mr. E. A. Thiele, Victoria.
	Mr. J. G. Tod, Darling Downs, Queensland.
	Mr. N. E. M. Walters, Canberra.
	Dr. W. H. White, University of Sydney.
Austria :	Dr. K. Lohwag, Professor in Plant Pathology, University College for Veterinary Science, Vienna.
Belgium :	Mr. K. L. J. Blommaert, Botanical Institute, Gent.
Brazil :	Mr. K. Hagelund, Plant Protection Ltd.
Canada :	Mr. W. Ferguson, Division of Horticulture.
	Dr. H. Hill, Division of Horticulture.
	Dr. J. C. Wilcox, Summerland Experiment Station, British Columbia.
Ceylon :	Mr. F. C. Cooke, Director, Coconut Research Institute, Dankotuwa.
	Mr. C. A. Loos, Tea Research Institute, Talawakelle.
	Dr. H. E. Young, Director, Rubber Research Institute.
Cyprus :	Mr. A. Papasolomontos, Agricultural Superintendent.
	Mr. D. Ramadan, Agricultural Department, Nicosia.
Denmark :	Mrs. Else Boken, Royal Veterinary and Agricultural College, Copenhagen.
	Mr. R. H. Jensen, Copenhagen.
	Mr. H. H. Rambusch, Royal Veterinary and Agricultural College, Copenhagen.
	Miss Anna B. Weber, Danish State Research and Experimental Station for Plant Diseases and Pests, Copenhagen.
East Africa :	Mr. E. G. P. Sherwood, African Explosives and Chemical Industries Ltd.
Finland :	Mr. T. J. Lapinleimu, Sulphuric Acid and Superphosphate Factories Ltd., Helsinki.
France :	M. S. Coppenet, Station Agronomique de Kerguelen, Quimper.
	Dr. G. L. Ducet, Centre Nationale de Recherches Agronomiques, Versailles.
	M. J. L. Dumas, Institut des Fruits et Agrumes Coloniaux, Paris.
	M. M. Trocme, Station Centrale d'Agronomie, Versailles.
Germany :	Dr. E. G. Brauckmann, Institut der Universität, Bonn.
	Dr. P. E. Frohberger, Farbenfabriken Bayer Biologischer Institut, Bayerwerk.
	Dr. P. Schwarze, Max Planck Institut, Hanover.
	Mr. J. R. G. E. Wehrmann, Institut fur Pflanzenernährung und Bodenkunde, Kiel.
Gold Coast :	Dr. S. N. Adams, Tafo.
Holland :	Mr. B. Buitendijk, Royal Netherlands Ministry of Agriculture, 'S-Gravenhage.
	Mr. J. Butijn, Laboratorium van Zeelands Proeftuin, Wilhelminadorp.
	Mr. P. J. Ente, University of Wageningen.
	Mr. G. A. M. A. van Gills, Royal Netherlands Ministry of Agriculture, 'S-Gravenhage.
	Mr. S. Samson, Institute for Soil Research, Groningen.
India :	Mr. W. Wight, Tocklai, Assam.
Iraq :	Mr. B. M. Yousif, Directorate General of Agriculture, Baghdad.
Israel :	Dr. R. Samisch, Hebrew University, Jerusalem.

Italy :	Miss Ornella Vergnano of Florence University.
Japan :	Professor Kinichiro Sakaguchi, Faculty of Agriculture, University of Tokyo.
Kenya :	Dr. R. W. Rayner, Coffee Research Station.
Malaya :	Mr. F. W. Hutchinson, Rubber Research Institute.
	Mr. Lin Ban Jee, Singapore.
New Zealand :	Mr. P. J. Clark, D.S.I.R., Auckland.
	Mr. D. V. Gordon, I.C.I. (New Zealand) Ltd.
	Mr. A. M. W. Greig, Director of Horticulture, Wellington.
	Mr. C. A. Ivory, Nelson.
	Mr. G. O. Ivory, Marlborough.
	Mr. A. C. Maisey, Nelson.
	Mr. J. Watt, Department of Agriculture.
Nigeria :	Mr. R. A. Fitzjohn, Medical Department.
	Mr. M. Greenwood, Agricultural Department.
Norway :	Mr. A. S. Bylterud, Norwegian Plant Protection Institute, Vollebekk.
	Mr. L. K. Foss, Ullensvang Research Station, Hardanger.
	Mr. K. Oland, Ullensvang Research Station, Hardanger.
	Mr. E. Vidvei, Horticultural and Agricultural Adviser, Sogndal.
Pakistan :	Dr. S. Hedayetullah, Director of Agriculture, East Pakistan.
South Africa :	Professor M. W. Black, Stellenbosch University.
	Mr. W. L. Hall, East Transvaal.
	Dr. S. Rehm, Division of Horticulture, Department of Agriculture, Pretoria.
	Mr. D. Roberts, Eastern Province.
	Professor H. Shaw, Director, Wattle Research Institute, Pietermaritzburg.
	Mr. Gwynne Thomas, Witwatersrand University.
Spain :	Dr. Guzman, University of Murcia.
Sudan :	Dr. W. S. Richards, Central Rainlands Research Station, Wad Medani.
	Mr. Peter Walton, Central Rainlands Research Station, Wad Medani.
Sweden :	Mr. Sven L. Jansson, Royal Agricultural College of Sweden.
Switzerland :	Dr. E. Bovay, Federal Station, Lausanne.
Tanganyika :	Mr. R. G. Tapley, Coffee Research Station.
Turkey :	Mr. N. Kucukkoca, Government Entomologist.
Uganda :	Mr. Acaye, Department of Education.
	Mr. A. Kamya, University of Bristol.
	Mr. W. R. Mills, Agricultural Department.
U.S.A. :	Dr. G. M. Darrow, Beltsville, Maryland.
	Professor W. L. Nelson, University of North Carolina.
	Dr. E. Trigg, Missouri.
	Mr. P. Smith, Maryland.
	Mr. R. Wiggam, West Grove, Pennsylvania.
Venezuela :	Dr. Baez, Agricultural Adviser, Shell Petroleum Company.
Yugoslavia :	Dr. D. Stankovic, University of Belgrade.

Shows and Demonstrations. Exhibits were staged at the following shows :—

Royal Horticultural Society, Chelsea—May 20th–23rd.
Three Counties Show, Worcester—June 10th–12th.
The Royal Agricultural Show—Newton Abbot, July 1st–4th.
Bristol Horticultural Show—September 3rd–5th.
The Brewers' Exhibition—Olympia, London, November 24th–28th.

Students and Voluntary Workers. The number of research students has again been severely limited owing to lack of laboratory bench space. This situation is greatly to be regretted since many applicants of graduate status have had to be refused. This unsatisfactory situation must, unfortunately, remain until new laboratories are built.

Mr. E. J. Skerrett left at the end of March after working with Dr. Woodcock for two years studying for his Ph.D. Degree, which has since been awarded.

Dr. S. C. Agarwala of the University of Lucknow, left the Station on 8th November after working with Dr. Hewitt for two-and-a-half years under a study-grant awarded by the U.P. Government, India, and a grant from the A.R.C. for the extended period beyond two years. He has been working on the role of molybdenum in the nitrogen and carbohydrate metabolism of the cauliflower and on the mode of origin of "Whiptail," and has since been awarded the degree of Ph.D. in the University for his thesis entitled " A Study of the Molybdenum Nutrition in Cauliflower."

Mr. S. T. C. Wright continued his work under Dr. Luckwill in the Pomology Section, as the holder of the Boots' Scholarship from 1951 to 1953.

Dr. E. Fernandez Caldas, holder of a scholarship of the Spanish High Council of Scientific Research for study in England, spent the period 21st April–25th June at the Station, studying methods employed for the use of radio-active phosphorus (P^{32}) in experiments on plant nutrition.

Mr. M. I. Candela Martinez, of the Instituto de Edafologia y Fisiologia Vegetal, Madrid, holder of a British Council scholarship, has been working since March 3rd under Dr. Hewitt's direction on a research project for a higher degree in the University.

M. G. L. Ducet, of the Centre National de Recherches Agronomiques, Versailles, spent the months of July and August working with Dr. Hewitt after attending the British Council Course. He hopes to return for a further period.

Mr. R. H. Jensen, post-graduate student from Copenhagen, undertook a period of voluntary work in Dr. Martin's laboratory from 2nd to 29th July, and Mr. P. J. Ente, a student from Wageningen University, Holland, spent the month of August in Dr. Bould's section working on analytical techniques.

Mr. J. C. Blore commenced the six-months award of the Memorial Studentship in Cider-making on 16th October.

The following courses were given in the Domestic Fruit and Vegetable Preservation Section :—

Preservation and Judging Preserves—July 14th–August 1st.

Preservation of Fruit and Vegetables—August 18th–30th.

Thanks are due to Mrs. Griffith for assistance given in the courses.

Social Activities. The Welfare Committee served during the year under the Chairmanship of Mr. N. G. Morgan. It was responsible, as usual, for the organization of the Christmas Party, which was held in the Village Hall on December 23rd, when over 240 members of staff and their friends enjoyed another happy evening, including a pantomime—" Cinderella "—written and produced by the staff.

During the year a staff Football Club was formed, which was elected to the third division of the local Church of England league and commenced play at the beginning of the 1952–53 season. Although only five matches were won out of the nineteen played, the team's record improved considerably towards the end of the season and they are looking forward to resuming play next year.

The Cricket Club continued during the 1952 season under the difficulties arising from lack of a ground of their own.

The Men's Tennis Team again played in the Bristol Tennis League, and were promoted to the next section. In spite of frequent flooding of the hard court, play took place whenever possible during lunch time and evenings, and it is hoped that the surface of the hard court may be repaired before next season.

Acknowledgments. We would wish to place on record our acknowledgments to members of the staff who have carried out special duties in addition to their normal work. Dr. Kearns has again given valuable assistance in connexion with the planning of new buildings and in organising and supervising the workshops, including repair work to equipment, machinery and tools for the laboratories and farm. Mr. Spinks has continued to act as Librarian. Mr. Morgan has filled the office of Chairman of the Welfare Committee this year, and in that capacity was responsible for organizing the Christmas Social. Miss Sturdy has again acted as Chairman of the Canteen Committee, and, together with Miss James, Miss Locke and Miss Moseley, has undertaken the catering for the Canteen and the arrangements for the Christmas Dinner, which was held on December 22nd in the Library, and was greatly enjoyed by a large number of staff.

Our grateful acknowledgments are due to these and to other members of staff who have helped in various ways during the year, and to the Assistant and Technical staffs and all other employees for their loyal support in carrying out the programme of research work.

We should also like to thank all our friends outside the Station, particularly growers and commercial firms, for their help and co-operation in our work during the year.

THE ESTABLISHMENT AND PERFORMANCE OF DESSERT APPLE TREES UNDER DIFFERENT SYSTEMS OF SPACING, PLANTING AND SHAPING.

By E. W. HOBBIS and E. CATLOW.

Introduction.

In the winter of 1947–48 an area of approximately 3¾ acres at Long Ashton was planted to dessert apple trees to be grown under different systems of spacing, planting and shaping. The following report concerns the performance of these trees during their first five years in the field, a period during which they have more or less completed their formative stage.

It was hoped, originally, to record the whole of the expenditure incurred in establishment, labour, manures, spray materials and maintenance for each system of planting, but this was found difficult to accomplish accurately due to the small areas involved and the difficulty of accurately assessing machine, sprayer and man-hour times incurred on many cultural operations.

Planting costs, however, were easily ascertained and these are given on a comparative acreage basis, together with an approximate of establishment costs in the first year.

Soil and Site. The field has a gentle slope to the south, with greater soil depth at the lower end. Drainage is generally good but previous experience in growing vegetables on this area had showed the fertility to vary considerably across the field as well as with the slope according to general soil depth.

The soil is derived from Keuper Marl with some overwash from the limestone and a considerable amount of stone is present both in surface and sub-soil.

Plot lay-out.

The four systems of planting were as follows :—

(a) Bush trees on M.II rootstock at 24 ft. square, i.e. 75 trees per acre, without undercropping.
(b) Bush trees as in (a) with the addition of dwarf " fillers," on M.IX rootstock interplanted at 12 ft. apart, i.e. 75 permanent trees, plus 227 " filler " trees per acre—302 trees in all.
(c) Single cordons on M.II rootstock, planted 8 ft. × 3 ft., i.e. 1,815 trees per acre.
(d) Dwarf pyramids on M.II rootstock, planted 8 ft. × 4 ft., i.e. 1,361 per acre (trees not staked).

These four systems were not replicated but were planted in single blocks running N. to S. with the slope of the land ; systems (a) and (b) covering some 1½ acres each and systems (c) and (d) approximately ⅓ of an acre each.

Materials for most of the trial were raised at the Research Station, excepting a small number of trees of Cox on M.IX which were supplied by East Malling Research Station and planted out as " maiden " trees. A selection of " maidens " was made in the nursery so that " well-feathered " trees were chosen for cordons and dwarf pyramids in particular.

Varieties and rootstocks. The greatest number of trees, for each system, were of Cox's Orange Pippin. In the bush tree plots Laxton's Superb and Winston were included as pollinators, the former variety forming "buffer" trees on the flanks and between the "open" and the "filler" plant. The planting was so arranged that five trees of Cox and one of Winston formed a unit for trees on M.II. In the cordon and dwarf pyramid sections Winston was used as a 1 in 4 pollinator in the row. M.II rootstock was used for all but the bush "filler" trees which, in view of their expected short tenure, were worked on M.IX.

Comparative planting costs.

The following table sets out the comparative costs per acre of planting and early establishment of dessert apples under the four systems in 1948, at the then ruling costs of labour and materials. No figure has been included for cost of land or for perimeter fencing. Since 1948 costs of labour and materials have risen but the price of trees has fallen. To these costs should be added those expenses incurred during the first season after planting out, as such operations as mulching, manuring, cultivations and spraying are inseparable from the planting and establishment.

TABLE I.

ESTABLISHMENT COSTS PER ACRE (£).

System	Permanent bush	Permanent bush + filler	Oblique cordon	Dwarf pyramid
Trees (at 5/- each)	19	75	454	340
Materials	6	24	142	—
Labour	12	17	100	22
Total	37	116	696	362

Management of each system.

(a) *Bushes at 24 ft. square.*

Apart from the fact that planting on the square is the simplest possible arrangement of trees in a bush plantation, the reason for using this planting distance was that past experience at Long Ashton has shown that Cox on M.II can utilise this spacing within 20 years of planting, leaving not more than tractor space between the approaching branch system.

Intercropping was avoided, to give the trees every opportunity to develop rapidly and fill out the spacing in the shortest possible time.

Pruning and shaping have aimed at producing trees of delayed-open-centre form, in which a central stem 4 5 ft. high carries branches from 2 ft. 6 in. upwards, and is terminated by an open-centre head.

The branches have been spaced in a roughly spiral arrangement up this central stem but have not been developed as opposed pairs as is sometimes recommended.

Tipping of leaders has been moderately light throughout and many trees have now reached a height at which a proportion of leaders cannot be reached easily from the ground. General policy has been to dispense with tipping once this stage is reached, thus avoiding an over-stiffening of the branches, and encouraging the tree to open outwards by early cropping and to form replacement shoots on the inner and upper sides. These replacement shoots and any other leaders within easy reach have been tipped more or less lightly according to position and vigour.

The treatment of laterals has followed the general principles of renewal pruning and by the end of the fifth year most trees have become well furnished with one-, two- and three-year-old laterals and a few older spurs. Development of the trees on the whole has been rapid and the following table shows the increase in total girth measurements from the time of planting, and height and spread measurements taken after winter pruning at the end of the fifth year.

In the third year a number of trees, mainly Cox, developed stem and crotch cankers. Some trees had to be replaced, whilst others, less seriously affected, recovered after paring and painting. In addition, where stem shoots were produced below a stem canker these were retained and grafted into the upper stem. This bridging of cankers has seemed worthwhile as a method of preserving valuable young trees.

(b) *Bushes with " Fillers."*

Trees on M.IX were chosen for the " fillers " in anticipation of their development and fruiting during the short period of years, perhaps 10, in which they could be allowed to remain without detriment to the more permanent bushes.

Pruning and shaping of the more permanent trees has followed exactly along the lines described in (a) but the " filler " trees have been treated differently.

Branching was started at 2 ft. from the ground and tipping has only been hard enough to stiffen the few initial branches and to encourage sufficient new growth. Trees on M.IX cannot be trained readily with a delayed-open-centre form and, generally speaking, each cropping has bent branches outwards from the centre at an early age. The girth measurements, height and spread figures of these trees are included in Table II.

Soil management. Both plots (a) and (b) have been treated alike. Spring and summer cultivations with spring-tined cultivators and harrows have been frequent up to the end of August, when cultivations have ceased and weed has been allowed to grow. The soil has then not been cultivated again until the following early spring. The winter weed cover has checked erosion and has eased considerably the work of pruning and spraying under wet winter conditions.

Manuring. All trees were well mulched with F.Y.M. in the spring following planting, and fertiliser applications have been at the rate of 2 cwt. superphosphate and 1 cwt. sulphate of potash per acre. In the spring of the fifth year, however, the fertilisers applied included nitrogen in anticipation of grassing down during the year. The actual applications were 3:3:2 cwts. per acre of N, P and K applied in early April. Both plots were sown to Kent

White Clover at 3 lb. per acre, in early August 1952, i.e. during the fifth year after planting " maiden " trees.

Spraying. A routine winter and spring-summer spraying programme has been applied including spraying against Codling Moth, which caused considerable loss of fruit from the first crop in the third year after planting.

TABLE II.

BUSH TREES.

MEAN HEIGHT AND SPREAD AT END OF FIVE YEAR PERIOD AND MEAN ANNUAL GIRTH INCREASE.

Systems of planting	(a) Permanent bush only				(b) Permanent bush + filler			
	Height ft.	Spread ft.	Initial girth cms.	Annual girth increase cms.	Height ft.	Spread ft.	Initial girth cms.	Annual girth increase cms.
Variety and Stock—								
Cox on II	8·0	7·4	3·5	3·0	7·8	7·2	3·3	2·8
Winston on II ..	8·3	5·5	3·4	2·8	7·7	5·1	3·4	2·6
Superb on II	9·3	7·9	3·6	3·5	7·4	6·3	3·7	2·6
Cox on IX					6·1	6·3	2·9	1·8
Winston on IX ..					6·5	5·3	3·0	1·8
Superb on IX ..					5·5	4·7	3·1	1·4

(c) *Cordons.*

The 560 cordons of Cox and Winston on M.II rootstock were planted at the same time as the bush trees, in rows 8 ft. apart and with the cordons 3 ft. apart in the rows. Each tree was planted against a cane secured at an angle of 45° to a substantial wire fence. A sharp difference in soil fertility occurs half way along the rows and this has materially affected the growth and performance of the cordons in each sector. The variety Winston occurs as every fourth tree in the rows as a pollinator.

TABLE III.

CORDON AND DWARF PYRAMID.

MEAN ANNUAL GIRTH INCREASE (CMS.).

System of planting	(c) Oblique cordon		(d) Dwarf pyramid	
Variety and stock	Initial girth	Annual girth increase	Initial girth	Annual girth increase
Cox on II	3·2	1·0	3·1	1·3
Winston on II ..	3·3	1·0	3·2	1·4

Pruning and shaping. In the hope of obtaining heavy crops within a few years of planting the cordons were not pruned on the orthodox spurring methods that restrict cropping to a narrow band along each cordon stem. In order to increase this cropping space about one-third of the lateral shoots growing outwards and sideways were allowed to develop as small cropping branches and to speed up flower bud development, they were tied down on a curve during the summer months.

The retention of some proportion of the laterals in this way was practised from the " maiden " stage, when some of the " feather " shoots were untouched at the first pruning.

Laterals not dealt with in this way have been either summer-pruned, or spurred during winter pruning or sometimes shoots have been cut clean away. The cordons on the S. or bottom end of the plot have grown vigorously, and as they had reached the top of the fence in the fourth year they were lowered to an angle of about 30° to the ground to allow of greater length. These trees have grown very vigorously each year.

TABLE IV.

YIELDS (FIELD CROP).

System of planting	(a) Permanent bush only	(b) Permanent bush + filler	(c) Oblique cordon	(d) Dwarf pyramid	Remarks
No. of trees/acre :—	75	75 + 227	1815	1361	
		(i) *Mean yields lbs./tree*			
1949 Cox		0·5	2·6	1·2	
Winston ..		0·7	3·8	1·2	
Superb ..		0·6	—	—	
1950 Cox	1·5	0·7 + 3·4	2·4	1·0	
Winston ..	1·9	2·5 + 5·3	2·4	2·4	
Superb ..	6·0	5·7 + 6·9	—	—	
1951 Cox	2·0	1·6 + 4·1	2·6	0·2	Pyramids in grass. Severe lime-sulphur damage at petal-fall
Winston ..	3·4	3·2 + 6·4	4·0	0·4	
Superb ..	3·4	3·2 + 4·2	—	—	
1952 Cox	1·5	3·1 + 4·3	1·5	1·0	
Winston ..	12·1	9·4 + 17·4	3·5	6·5	
Superb ..	21·3	24·3 + 21·5	—	—	
Total Cox	5·0	5·4 + 12·3	9·2	3·4	
Winston ..	17·4	15·1 + 29·8	13·8	10·5	
Superb ..	30·7	33·2 + 33·2	—	—	

A detailed account of the pruning and development of these cordons appeared in the R.H.S. Fruit Year Book for 1951–52 (1). Since that account appeared, however, it has become apparent from the crop picked during 1952 that, although total weight of crop was good, fruit colour that year suffered severely where shading and heavy cropping occurred.

Soil management. Cultivations given to the cordons have been similar to those given to the bush trees, although hand hoeing of the tree rows has, of course, been necessary for a width of about 18–24 in. on either side.

Manuring, too, has been the same as for the bush trees, with the addition of surface mulching for two seasons after planting over the whole plot and for each season since for the upper or poorer half of the plot.

Grassing down of the alleyways was done with Kent White Clover in August in the fifth year after planting, but a ribbon of soil was kept hoed and free of weeds and grass along the tree rows.

(d) *Dwarf pyramids.*

The trees for this plot were planted on a tapering piece of land alongside the other plots and in line with the cordons in rows 8 ft. apart with trees 4 ft. apart in the rows. This distance keyed in very well with the width of the cordon rows and seemed an appropriate spacing for the use of small mechanical tools for cultivations and spraying, whilst retaining as high a tree population as possible.

The varieties Cox and Winston were planted at the ratio of 3:1 as with the cordons, and all trees were worked on M.II rootstock. The trees were not staked or otherwise supported.

Pruning and shaping. "Well-feathered" maiden trees were chosen for planting. Each leading shoot was headed off at about two feet from the ground and much of the "feather" was used to form initial small branches in the trees whilst others formed the earliest cropping wood.

No summer pruning was done during the first year after planting out and winter pruning consisted of shortening the leader to approximately 12 in. in length and tipping some of the laterals to form small branches.

Manuring. Manurial treatment was the same as for the bush trees until the second season after grassing down, when 3 cwt. per acre of nitro-chalk was given in addition to superphosphate and potash.

Grassing down. During the second season, growth was very vigorous and some summer pruning was done to reduce vigour. As this vigorous condition was repeated in the third year the plot was grassed down to Cotswold White Clover and Timothy S.50 in August in order to check vigour, and no nitrogen was applied prior to grassing down.

As with other areas in the same field, the soil fertility pattern varies markedly and in an erratic manner and this has resulted in some vigorous trees whilst others have not grown satisfactorily. The general standard of the dwarf pyramids, however, at the end of the fifth year was satisfactory, following on two seasons of a generally drastic check following the grassing down. There was a severe drop of young fruits after the 2nd post-blossom lime sulphur spraying in the 4th and 5th years, a happening perhaps related to the low nitrogen status of the trees in these two seasons following an over-vigorous condition previously.

Cropping.

The varieties Cox and Winston are included throughout each system of planting and the figures for Superb are also given.

The mean crop per tree is lower than expected, perhaps partly due to fruit drop in Cox following a combined effect of grassing down and lime-sulphur damage, and to size reduction in Winston in the last season.

The cropping capacity to date of Winston has been outstanding. This variety forms flower buds freely at the tops of new shoots, on laterals and also on self-formed spurs. The fruit then tends to set in bunches, however, and this necessitates thinning in order to obtain fruits of marketable size.

Superb has cropped erratically from year to year and fruit size has been somewhat large.

The following tables set out the crop in pounds per tree and the grading of the crops which have been gathered from the three varieties growing under the four systems of planting.

TABLE V.

FRUIT QUALITY.

	(a)	(b) Bush	 Filler	(c)	(d)	Remarks
	(ii) " *Extra Fancy* " *and* " *Fancy* " *Grades* as percentage of field crop					
1949 Cox	—	—	not graded	75	56	
Winston ..	—	—		—	—	
Superb ..	—	—		—	—	
1950 Cox	74	70	78	90	73	
Winston ..	67	62	74	80	61	
Superb ..	67	61	66	—	—	
1951 Cox	60	57	51°	74	38^+	+ poor size
Winston ..	84	88	79	81	70	° splitting of skin
Superb ..	55	67	53°	—	—	
1952 Cox		70	76	59*	81	(a) and (b) graded as one unit
Winston ..		85^+	85	58^{+}*	77^+	+ poor size
Superb ..		77	71	—	—	* poor colour

Comparison of all four sets of results serves no useful purpose, but valuable comparisons can be made between the results obtained from the " open " and the " filler " bush planting and also between the cordons and the pyramids.

Because of circumstances already stated the results for the dwarf pyramids have been disappointingly low but the trees are now in a state of balance between wood growth and flower bud and should perform satisfactorily in future. Sowing down of the whole area to Wild White Clover was completed in August 1952, five years after the original planting.

PLATE I

Development of Cox's Orange Pippin on MII rootstock : pruned tree in successive years.

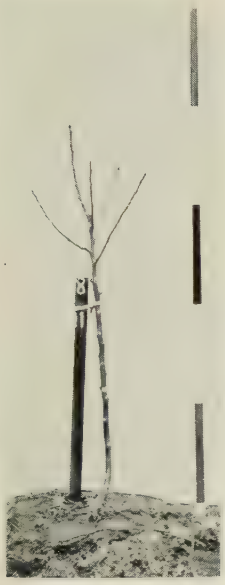

Fig. 1. After first year.

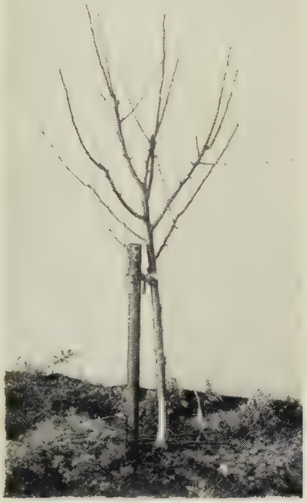

Fig. 2. After second year.

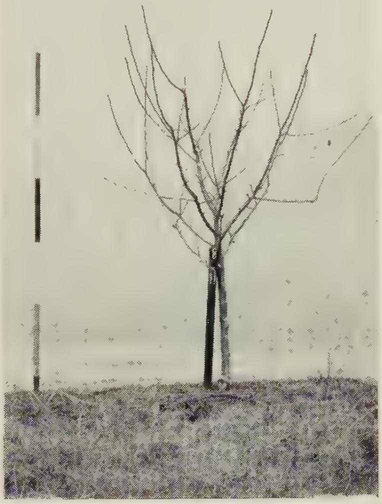

Fig. 3. After third year.

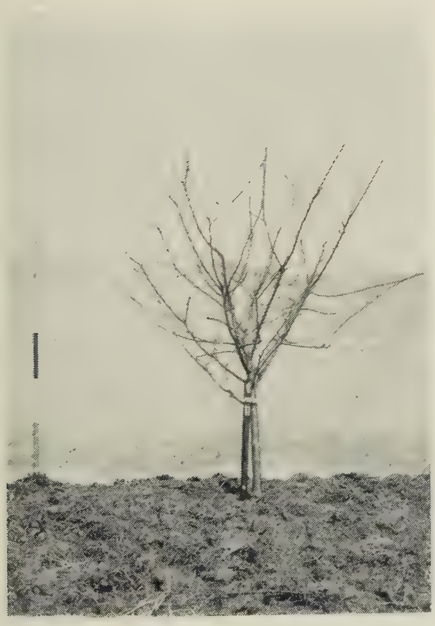

Fig. 4. After fourth year.

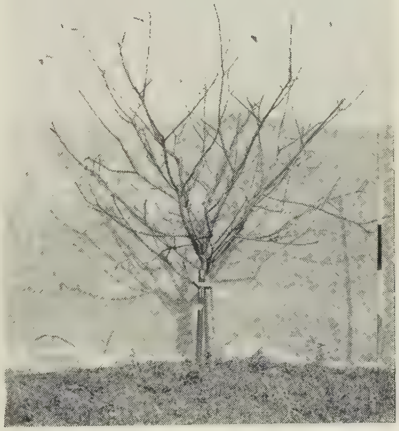

Fig. 5. After fifth year.

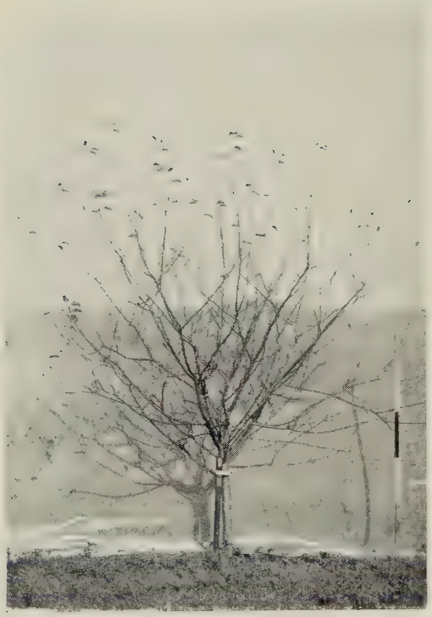

Fig. 6. Cox's Orange Pippin on MII rootstock. Before pruning, five years after planting as a maiden.

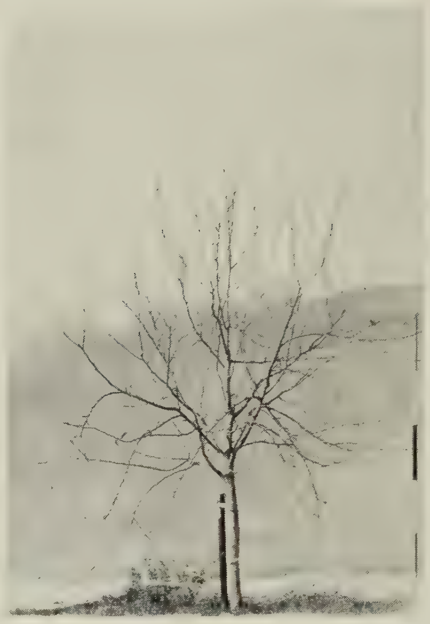

Fig. 7. Laxton's Superb on MII rootstock. Before pruning, five years after planting as a maiden.

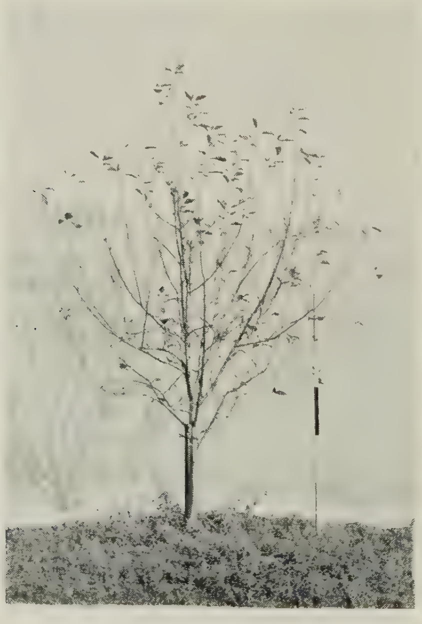

Fig. 8. Winston on MII rootstock. Before pruning, five years after planting as a maiden.

Figures 1 to 5 published with this report show the development and pruning of a bush tree of Cox on M.II, from the first to the end of the 5th year after planting out. The rapid development of the branch system and the volume of the head obtained in this period is clearly shown.

Figs. 6, 7 and 8 show trees of Cox, Superb and Winston on M.II at the end of the same period, but before being pruned.

REFERENCE.

(1) Hobbis, E. W. (1951). A method of pruning and training cordon apple trees. *Fruit Year Book*, **5,** 44–48.

A NURSERY METHOD DESIGNED TO REDUCE THE ATTACKS OF BACTERIAL CANKER ON THE STEMS AND CROTCHES OF YOUNG PLUM TREES.

By E. W. HOBBIS and A. I. CAMPBELL.

A field trial was laid out at Long Ashton in October 1952 to study the resistance or susceptibility to Bacterial Canker infection of four varieties of plums : Czar, Victoria, Warwickshire Drooper and Giant Prune ; low and high worked on Myrobalan " B " rootstock.

The growing of material for this trial provided an opportunity of investigating a nursery method that would avoid all pruning and other knife operations during the winter months, a period when the spread of Bacterial Canker is likely to be most active, and when new pruning cuts would be most easily open to infection.

Myrobalan " B " was chosen as the rootstock for this trial because it is reputed to be resistant to Bacterial Canker, and is also a vigorous grower, capable of forming a straight, strong stem with wide crotch angles suitable for high-working.

Nursery methods.

In the winter of 1948–49, one-year-bedded stocks of Myrobalan " B," raised from cuttings, were planted in nursery rows and allowed to grow on untouched throughout the following year. In early spring 1950 the stocks intended for high working were cut down to about 1 in. from soil level. This resulted in two or three young shoots growing out from the base of the cut-down stock and when these were a few inches long the best shoot was selected and tied to a cane. The remaining shoots were then pinched out, as shown in Plate III, Fig. 1.

Concurrently with this operation a similar number of stocks were lightly tipped and left for the normal basal budding in July, to provide a series of low-worked trees of the same age, necessary to complete the trial.

Each selected basal shoot of the rootstocks to be run up for high working was tied every few weeks and growth was so rapid that by mid-July these shoots had reached a height of 5 ft. as shown in Plate III, Fig. 2. The top was then cut back to 4 ft. 6 in. to encourage the formation of lateral shoots. These quickly developed and when a few inches long, four or five well-spaced laterals were selected and the remainder were rubbed out. The topmost lateral tended to continue upwards, making a well-shaped head of branches (Plate III. Fig. 3).

Generally speaking the break of " feather " all down the stem was profuse, and these shoots were kept cut back to 5 or 6 in. throughout the summer and only the selected shoots were allowed to grow freely.

By early September 1950 the selected lateral shoots had grown 2 ft. or more in length, and they were then cut back to 15 in. from the main stem in order to reduce wind damage during the winter months. No further cutting was done until the late spring of 1951.

High working.

Prior to budding in July 1951 the laterals again made vigorous growth and the side shoots were stopped at 6 in. at intervals during the summer in order to prevent overcrowding of the nursery rows. Buds were inserted one per lateral with four or five on each head. The buds were placed on the sides of the laterals and about 6 in. from the main stem. The centre shoot was budded on the side facing into the prevailing wind, to help improve the shape of the head.

In the following year, 1952, the budded stock shoots were cut back or " snagged " as the nurseryman calls it, to approximately 6 in. beyond the scion buds in early April. Stock buds broke freely and were kept cut back until the scion buds showed signs of " breaking " (see Plate IV, Fig. 1) It was noted that the variety Warwickshire Drooper started into growth much more quickly than the other varieties, whilst Victoria was relatively slow and in several cases the buds failed to develop until the stock " snag " had been cut back close to the bud. The early bud growth of Warwickshire Drooper was not an advantage since a sharp May frost killed and distorted the growing points of some of the shoots.

The young " scion " shoots were tied to the stock " snag " as soon as they were 3 or 4 inches long (see Plate IV, Fig. 2). This was not only to prevent blowing out but also to keep the young scion shoots growing in an outward direction parallel to the stock laterals. Previous experience had shown that if the young scion shoots were not tied down they grew vertically and produced badly positioned initial branches.

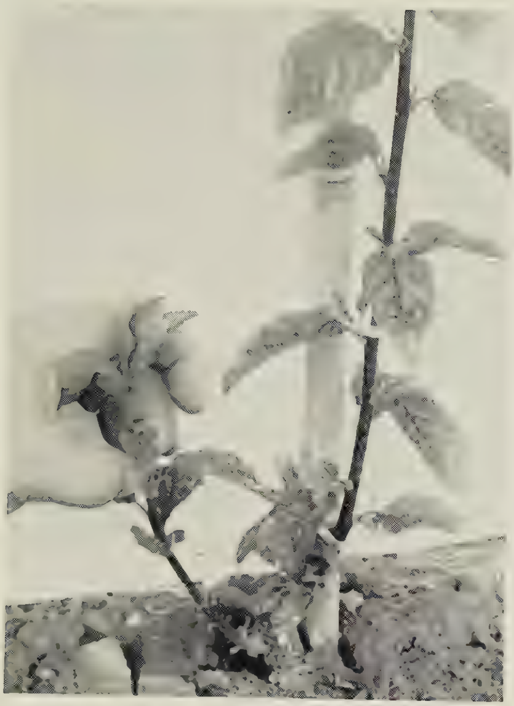

Fig. 1. Selected shoot tied in.

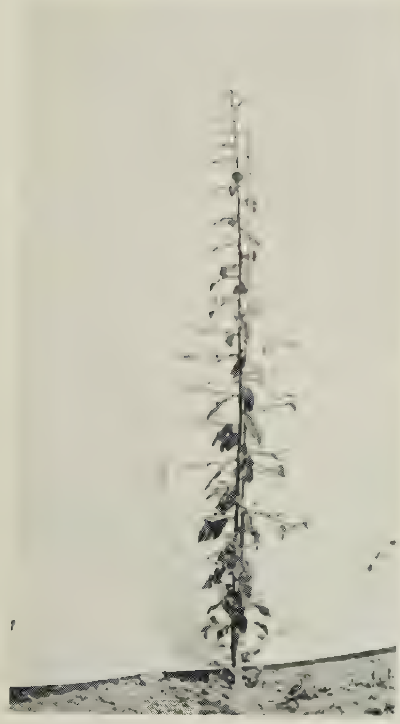

Fig. 2. Stock shoot, 5 ft. high, in July.

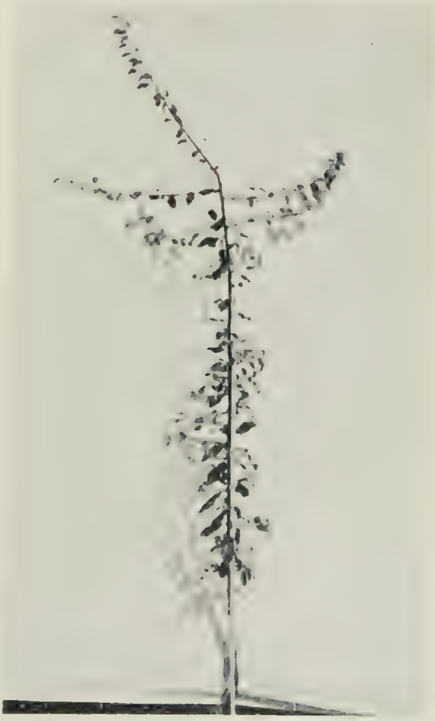

Fig. 3. Stock shoot in September. Note wide angle of lateral shoots.

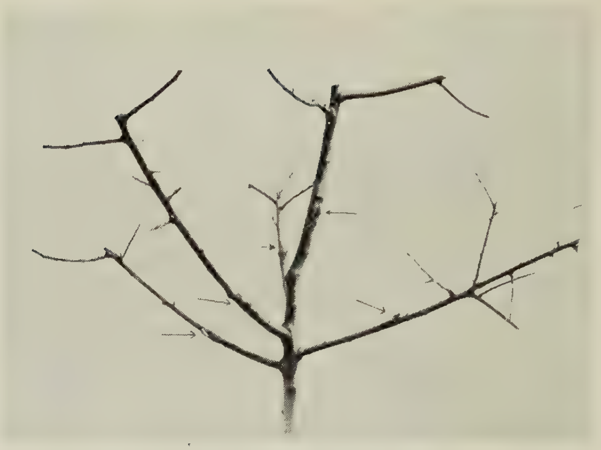

Fig. 1. Arrows indicate position of scion buds.

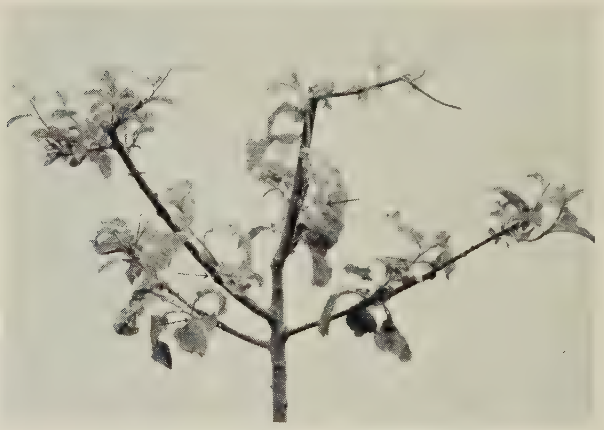

Fig. 2. Arrows indicate position of scion shoots.

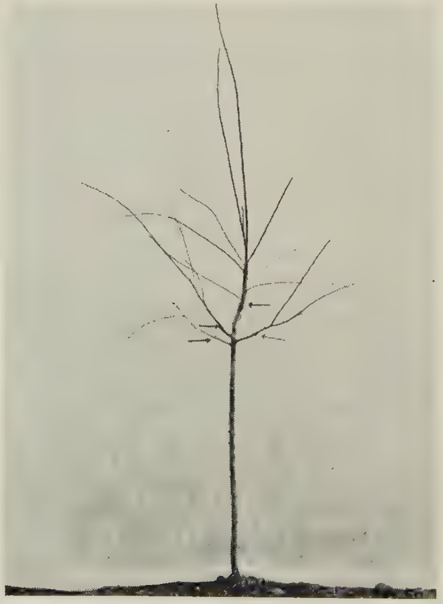

Fig. 3. A typical one-year-old head of Warwickshire Drooper, produced by the method described. Arrows denote position of scion buds.

Once the scion shoots were well established all stock growth was removed with the exception of one or two shoots at the end of the " snag " which were left to keep it alive. During this time the main stem of the trees was gradually cleared of all " feather " shoots. By early July the scion shoots were generally about 2 ft. in length, and as this was well past the end of the " snag " and since support could no longer be given, they were shortened back to about 14 inches to conveniently placed buds. This " stopping " helped to make the young scion shoots firm-up at the base so that during the latter half of July and early August the supporting " snag " could be cut out.

Secondary growth had reached 2–3 ft. by mid-September, the highest placed bud in all varieties being generally the most vigorous. Weaker scion shoots which had not reached 2 ft. by mid-July were not stopped, since the resulting secondary growth would have been produced too late to mature by the Autumn. On the other hand Warwickshire Drooper and Giant Prune had grown very vigorously and in order to reduce strain on the bud unions during gales, overlong secondary shoots were shortened back at the end of September, well before leaf-fall.

By this means it was possible to complete all the pruning operations during the growing months of the year. By pruning the primary branch shoots during mid-summer it was also possible to produce early secondary growth and form a well balanced shapely head which had the appearance of being 2 years old at the end of one season (see Plate IV, Fig. 3). In normal nursery practice the " bud " shoots might have grown 6-8 ft. during the year, growth which would largely have been pruned away after planting out.

It is not claimed that this method of producing young plum trees is likely to appeal to nurserymen, because it necessitates extra labour, but it seems a useful method whereby high-worked trees of excellent quality can be obtained. It may also be useful for a variety such as Early Laxton which is particularly susceptible to Bacterial Canker in the stem of the young nursery tree.

Low-worked trees.

Low-worked trees, being raised at the same time for inclusion in the trial, were also subjected to pruning operations which were carried out in the late Spring, Summer and early Autumn months only. The Myrobalan " B " rootstocks planted in winter 1948 49 were allowed to grow on untouched until the summer of 1950 when they were budded in the normal way about 4 in. above ground level. The stocks were shortened back to about 2 ft. 6 in. in the Autumn to avoid winter rocking.

In spring of 1951 the stocks were " snagged " back to about ½ in. above the bud. Subsequent vigorous growth was tied to a cane at frequent intervals and the total growth of the young trees during the year was between 6 and 8 ft.

In late March 1952 the maiden trees were headed-off at 4 ft. 6 in. from ground level, and later four or five well spaced shoots were selected to form the head of the tree. These made strong growth and were cut back to conveniently placed buds 12–14 in. from the main stem during June and early July. The secondary growth which was produced during July and August reached approximately 2–3 ft. by the end of the growing season and gave the young trees the appearance of being three years old instead of two.

The following table records the mean spread and the mean girth at 2 ft. from soil level of trees raised by the two methods described.

Mean spread in cm. of 38 trees			Mean girth in cm. of 38 trees		
Trees worked	High	Low	Trees worked	High	Low
Victoria	113	119	Victoria	76	64
Czar	77	73	Czar	74	67
Giant Prune	63	68	Giant Prune	75	69
Warwickshire Drooper ..	137	115	Warwickshire Drooper ..	85	77

The high-worked trees show stouter stems: the measurements of branch spread exhibit no consistent relationship with method of working.

GROWTH SUBSTANCES AS FRUIT-THINNING AGENTS FOR APPLES AND PEARS: PROGRESS REPORT.

By D. L. ABBOTT.

Introduction.

Experiments were carried out at Long Ashton in 1950 and 1951 to investigate the use of sprays of 1-naphthalene acetic acid and other growth substances as fruit thinning agents for apples and pears (1).

Of the substances tested, 1-naphthalene acetic acid (NAA) showed considerable promise as a thinning agent for apples, but it was found that the spray must be applied within 7–10 days of petal-fall if a reduction in fruit size was to be avoided. It was concluded that different varieties show marked differences in response to NAA and that experience must be gained over a number of years before recommendations for general use could be made.

The two varieties of pears tested seemed to respond differently from apples, treatment with NAA resulting in an apparent increased, rather than decreased, fruit-set.

The present paper gives the results of further experiments carried out in 1952 which were designed to investigate :—

(a) The effect of NAA sprays of different concentrations, applied approximately one week after petal-fall, on the fruit-set of three varieties of apple.

(b) The effect of NAA and 2-naphthoxy-α-propionic acid on the yield of four varieties of pear.

Fruit-thinning Trials with Apples.

Method.

36 uniformly large branches of mature Miller's Seedling bush trees were selected and sprayed with NAA at concentrations of 5, 10, 15, 20 and 30 p.p.m. or left unsprayed as controls. Thus each treatment was replicated six times. 48 branches of both Edward VII and Crawley Beauty were similarly selected and treated to give 8 replicates per treatment. The NAA was applied as an aqueous spray to the run-off point and, where necessary, spray drift was prevented by the use of a screen. Wetting agents were not used.

Each variety was sprayed 8 days after petal-fall. The weather was warm with long sunny periods during treatment of the Miller's Seedling and Crawley Beauty, but was cool and overcast when the Edward VII were sprayed.

TABLE I.

EFFECT OF SPRAYING THREE VARIETIES OF APPLE WITH VARIOUS CONCENTRATIONS OF NAA AT 7 DAYS AFTER PETAL-FALL.

Variety	Conc. of NAA p.p.m.	Fruits matured per 100 blossom clusters	Mean fruit weight (gms.)
Miller's Seedling ..	Control	65 (61)	54 (39)
	5	52	60
	10	**43**	61
	15	**42**	56
	20	52 (27)	56 (51)
	30	**44**	52
Edward VII	Control	58	134
	5	54	154
	10	43	146
	15	45	135
	20	**42**	126
	30	**37**	125
Crawley Beauty ..	Control	62 (63)	67 (72)
	5	**28**	75
	10	**25**	**82**
	15	**19**	**87**
	20	**17** (15)	**89** (90)
	30	**12**	**98**

Figures in Bold Type are significantly different from the controls at 5% level of probability.
Results in the 1950 trials are shown in brackets.

Results.

The results shown in Table I confirm the fruit-thinning effect of NAA, and difference in response between varieties. However, a comparison between the results of this experiment and the 1950 trial shows that whereas there is good agreement in the effect on Crawley Beauty, the thinning effect on Miller's Seedling was considerably greater in 1950 than in 1952. This may reflect a difference in the nutritional status of the trees in the two years, since although fruit-set was almost the same, the mean fruit weight on the control branches was only 39 gms. in 1950 while in 1952 it was 54 gms. This higher nutritional status was probably the result of thinning alternate trees in the winter of 1951. These results suggest that NAA is more effective as a thinning agent when the tree is relatively low in foodstuffs and competition between fruits consequently great.

Although considerable thinning, by at least some treatments, was achieved in all the three varieties, a significant increase in mean fruit weight was obtained only for Crawley Beauty. However, such was the extent of the thinning in this variety that, in spite of an increase in the *proportion* of marketable fruit (2″ or more in diameter) on the treated branches, the actual *number* of marketable fruit was greater on the control branches (Table II). Since the primary object of fruit-thinning is to increase not only the proportion but also the actual number of marketable fruit, these results appear disappointing. However, further work with other varieties must be done before a final conclusion can be reached.

TABLE II.

CRAWLEY BEAUTY. DISTRIBUTION OF FRUIT INTO SIZE GROUPS.

(No. of fruit per 100 blossom clusters).

Treatment	<1¾″	1¾–2″	2–2¼″	2¼–2½″	>2½″	Total	
						Unmarketable (<2″)	Marketable (>2″)
Control	3	14	19	19	7	17	45
5 p.p.m. NAA ..	1	4	7	10	6	5	23
10 ,, ,, ..	0	2	6	12	5	2	23
15 ,, ,, ..	0	2	4	8	5	2	17
20 ,, ,, ..	1	2	3	6	5	3	14
30 ,, ,, ..	0	0	1	5	6	0	12

Trials with Growth-Substances on the Yield of Pears.

Method.

50 uniform branches were selected from mature bush trees of each of the varieties : Williams, Laxton's Superb, Beurré Bedford and Conference. Treatments were an aqueous spray of NAA at 20 p.p.m. and of 2-naphthoxy-α-propionic acid (2-NOP) at 50 p.p.m. applied to run-off point. No wetter was used. The sprays were applied at full-bloom and at 7 days after petal-fall.

Thus each treatment was replicated ten times, the remaining ten branches of each variety being left unsprayed as controls.

Results.

As with apples, the pear varieties showed considerable differences in response (Table III). Williams was thinned by the 7-days-after-petal-fall application of both substances, while Conference was thinned by the 7-days-after-petal-fall application of NAA only. This is contrary to the results of the 1950 trial when both these varieties seemed to respond by an increased set following treatment with NAA at 20 p.p.m. Blossom was particularly abundant in 1952 however and the control branches carried about double the 1950 crop. This may have some bearing on the different effect of treatment between the two years. The fruit set of Superb and Beurré Bedford was not significantly affected by any treatment.

Mean fruit weight does not appear to have been affected by treatment, except for a decrease for Superb following NAA at 7 days after petal-fall, and an apparent increase for Beurré Bedford as a result of all treatments. However it is felt that the latter must be interpreted with some caution since it seems more likely that, for some reason, the control is significantly lower than the population mean weight.

TABLE III.

EFFECT OF GROWTH-SUBSTANCES ON FRUIT-SET AND FRUIT SIZE IN PEARS.

Treatment	Control	Full-bloom		Petal-fall + 7 days	
		NAA 20 p.p.m.	2-NOP 50 p.p.m.	NAA 20 p.p.m.	2-NOP 50 p.p.m.
	Fruit matured per 100 blossom clusters				
Williams	42	32	29	**29**	**26**
Laxton's Superb	60	52	46	54	63
Beurré Bedford	28	25	27	25	23
Conference	28	36	20	**12**	22
	Mean fruit weight (gms.)				
Williams	118	117	126	117	111
Laxton's Superb	109	109	110	**88**	107
Beurré Bedford	131	**153**	**158**	**169**	**157**
Conference	152	156	160	148	156

Figures in Bold Type are significantly different from the controls at 5% level of probability.

The results suggest that the reaction of pears to growth-substances applied as thinning agents is similar to that of apples, but that pears are less sensitive to treatment. Further experiments with higher concentrations will be necessary before a final assessment can be made.

Summary.

(1) 1-naphthalene acetic acid was applied 8 days after petal-fall at concentrations of 5–30 p.p.m. as a thinning agent to three varieties of apple.

(2) Miller's Seedling and Edward VII responded with a reduced fruit-set at the higher concentrations. Crawley Beauty was considerably thinned at all concentrations.

(3) An increase in mean fruit weight was obtained only with Crawley Beauty. So great was the degree of thinning, however, that the marketable crop from the treated branches was less than that from the controls.

(4) Four varieties of pear were sprayed with 1-naphthalene acetic acid and 2-naphthoxy-α-propionic acid at full bloom and 7 days after petal-fall. Contrary to the results of the 1950 trials, some reduction of fruit-set was obtained with Williams and Conference, but further trials will be necessary to confirm the thinning effect of growth-substances on pears.

Acknowledgments.

Thanks are due to Dr. L. C. Luckwill for his suggestions and advice in planning these experiments, and to Miss A. J. Beagles for assistance in spraying and recording.

REFERENCE.

(1) Luckwill, L. C. (1951). *Ann. Rept. Long Ashton Res. Sta.*, 1951, 33.

POLLINATION REQUIREMENTS OF CIDER APPLE VARIETIES.

PROGRESS REPORT, 1952.

By R. R. WILLIAMS.

Most dessert and culinary varieties of apples are, to some extent, self-sterile and set a heavier crop if provision for cross-pollination is made. The same is true of cider apples. The traditional method of growing cider fruit, as seen on many West-country farms, is in a standard orchard containing a large number of varieties, affording ample opportunity for cross-pollination. Many of these varieties however give a low yield or produce fruit of poor vintage quality, and consequently are no longer being planted. In the more recently-established cider orchards and bush plantations the varieties chosen are restricted to the few of known merit. This trend has accentuated the need for knowledge of the effects of pollination factors on the cropping of cider varieties.

Except for the preliminary observations by Spinks (1930), little work has been done in determining self-fertility or self-sterility of cider apple varieties ; so experiments were started at Long Ashton in 1949 to investigate pollen viability and pollination requirements of a number of the better-known varieties.

Pollen viability.

Tests were made of the germinative capacity of pollen of numerous cider varieties, with the aim of selecting the most suitable pollens for cross-pollination. Flowers for these tests were collected just after the petals had unfolded, at the stage when the anthers were mature but the pollen sacs unruptured. Pollen from younger or older flowers was usually less viable. The blossoms were spread on trays and left in a warm room overnight for the anthers to dehisce. Germination tests were made the following morning, although it was found that the supply of pollen from the dried flowers was usually maintained over several days without loss of viability. The pollen was germinated in a hanging-drop culture of sucrose solution kept at 25° C. for 2 hours. As the percentage germination was affected by the density of the pollen suspension, the sowings had to be reasonably uniform.

To obtain maximum germination it was necessary to use different concentrations of sucrose for pollens of different varieties : a 5 per cent solution proved best for Yarlington Mill, 10 per cent suited the majority of varieties, but 20 per cent was necessary for Kingston Black. In sucrose concentrations below 20 per cent many of the pollen tubes of Kingston Black burst soon after germination : in concentrations over 20 per cent the rate of germination was retarded.

Samples normally showed maximum germination in 2 hours ; if observation was delayed after this period the growth of the pollen tubes impeded counting. Each test was made in triplicate and three microscope fields (300-450 grains) were counted for each culture. The pollen from some varieties germinated poorly after wet or cold weather : consequently several tests were needed to determine the maximum germination rate. Reduced viability caused by unfavourable weather could often be anticipated because it was accompanied by irregular dehiscence of the anthers.

Experimental data and comments.

It was soon noted that varieties fell into two sharply-differentiated groups. Germination rates were either between 0 and 30% or between 70 and 100%. Only one variety examined has consistently given an intermediate figure. The results are summarised in Table I. Germination figures have been grouped as follows :—

Poor	$<30\%$.
Medium	30–70%.
Good	$>70\%$.

The varieties Broadleaf Jersey, Bulmer's Norman, Butleigh 12, Court Royal, Crimson King, Gascoynes Scarlet, Godard, Improved Pound, Morgan Sweet, Sherrington Norman, Strawberry Norman and Tom Putt all produced poor pollen. Examination of the chromosome constitution of Strawberry Norman confirmed that this variety was a triploid. Further observations on the chromosome complement of varieties regularly producing poor pollen will be reported later.

Two of the varieties examined, Eastham and Cherry Norman, were morphologically male-sterile. Flower development varied from forms containing vestigial anthers only to perfect flowers with anthers containing viable pollen. The pollen sacs shrivelled up when the flower opened and were never observed to dehisce. The blossom period of both these varieties is late or very late, and it is of interest to note that in one of the cider orchards containing no other late-flowering varieties, there has been no recorded crop of fruit in the past four years from the Eastham or Cherry Norman varieties, which are in adjacent rows.

Self- and cross-pollination experiments.

Method.

All pollinations have been carried out in the field on standard trees or bush trees at Long Ashton. The standard cider orchard is now fifty years old, while the bush plantation was established in 1933. Better results were usually obtained in the bush plantation because pest control was more efficient than that obtained on the larger standard trees.

Flowers from the pollinator variety under trial were collected and treated as for a pollen viability test. Pollinations were usually carried out the following day. The germination rate was tested in the morning and, if this proved satisfactory, the pollen was used during the afternoon.

The blossom clusters to be pollinated were selected from those with at least four lateral flowers in the late balloon stage. Four of these lateral flowers were emasculated and then pollinated by lightly brushing the stigmas with a dried pollinator flower. The rest of the blossom cluster, including the terminal flower was then removed and the cluster enclosed in a Pergamine bag. The bags were removed when the flowers had passed the pollinating stage. The number of developing fruitlets was counted before and after the June drop.

The varieties Dabinett, Michelin and Sweet Coppin were self-fertile. Stoke Red may also belong to this group. All four varieties however showed a heavier set of fruit when cross-pollinated by a variety producing good pollen.

In addition to the suspected triploid, Bulmer's Norman, the varieties Backwell Red, Sweet Alford and Yarlington Mill appeared to be self-sterile.

Varieties producing good pollen have always proved successful pollinators when tried but it is not yet possible to say whether any particular pollinator is better than another for inducing heavy yields in a given variety. The number of pollinations was too small to make comparisons, for pests such as sucker must have frequently affected results.

Summary.

Pollen viability tests have been carried out with seventy-four varieties. Twelve varieties, Broadleaf Jersey, Bulmer's Norman, Butleigh 12, Court Royal, Crimson King, Gascoynes Scarlet, Godard, Improved Pound, Morgan Sweet, Sherrington Norman, Strawberry Norman and Tom Putt have consistently given low germination rates and will obviously be poor pollinators. Two varieties, Eastham and Cherry Norman, are morphologically male sterile.

Bulmer's Norman, as expected, would give no set when selfed but when crossed with a suitable pollinator the yield was usually high.

Dabinett, Michelin and Sweet Coppin are self-fertile, but give higher yields when cross pollinated.

Yarlington Mill, Backwell Red and Sweet Alford have failed to set fruit when self-pollinated. Those varieties producing a high percentage of viable pollen when used as cross pollinators have all proved successful.

TABLE I.

POLLEN VIABILITY.

Variety	Germination	Variety	Germination
Ashton Brown Jersey	Good	Joeby Crab	Good
Ashton Red	Good		
		Lady's Finger	Good
*Backwell Red	Good	Lambrook Pippin	Good
Bedan des Partes	Good	Langworthy	Good
Belle Norman	Good		
Broadleaf Norman	Poor	Magg's Seedling	Good
*Bulmer's Norman	Poor	Major	Good
Butleigh 12	Poor	Médaille d'Or Spreading	Good
Butleigh 21	Good	Médaille d'Or Upright	Good
		*Michelin	Good
Cap of Liberty	Good	Morgan Sweet	Poor
Cherry Norman	Good†		
Cherry Pearmain	Good	Neverblight	Good
Chisel Jersey	Good	Northwood	Good
Coleman's Seedling	Good		
*Court Royal	Poor	Red Foxwhelp	Good
*Crimson King	Poor	*Red Jersey	Good
		Red Norman	Good
*Dabinett	Good	*Reine des Pommes	Good
Doux Amer	Good	Reinette Obry	Good
Dove	Good	Rouge Bruyêre	Good
Dufflin	Good	Royal Wilding	Good
Dymock Red	Good		
		Sharp's Summer	Good
Eastham	Poor†	Sherrington Norman	Poor
Eggleton Styre	Good	Silver Cup	Good
*Ellis Bitter	Good	Slack Ma Girlde	Good
		*Stoke Red	Good
Fair Maid of Devon	Good	Strawberry Norman	Poor
*Frederick	Good	*Sweet Alford	Good
Fréquin Audièvre	Medium	*Sweet Coppin	Good
Gascoynes Scarlet	Poor	*Tardive Forestier	Good
Gatcombe	Good	Tom Putt	Poor
Godard	Poor	*Tremlett's Bitter	Good
Hangdown	Good	Upright Styre	Good
Harry Master's Jersey	Good		
		White Alphington	Good
*Improved Foxwhelp	Good	Woodbine	Good
Improved Pound	Poor		
		Yarlington Mill	Good
*Kingston Bitter	Good	Yellow Styre	Good
*Kingston Black	Good	Yeovil Sour	Good
Knotted Kernel	Good		

*Tests carried out over more than one season. †Pollen not released from the anthers.

TABLE II.

RESULTS OF SELF- AND CROSS-POLLINATIONS.

Varieties	Year	Flowers pollinated	Initial set	% initial set	Fruit matured	% fruit matured
Dabinett Selfed	1949	220	50	23	26	12
Dabinett Selfed	1950	216	178	83	37	17
Dabinett Selfed	1952	40	10	25	2	5
Dabinett × Michelin	1952	40	31	78	20	50
Dabinett × Improved Foxwhelp	1952	40	40	100	38	95
Dabinett × Yarlington Mill	1952	60	42	70	30	50
Michelin Selfed	1952	40	36	90	31	78
Sweet Coppin Selfed	1950	208	81	39	27	13
Stoke Red Selfed	1949	224	28	13	3	1
Stoke Red Selfed	1950	224	66	30	5	2
Stoke Red Selfed	1951	160	16	10	4	3
Stoke Red × Dabinett	1951	48	47	98	25	52
Stoke Red × Sweet Coppin	1951	48	45	94	21	44
Stoke Red × Red Jersey	1951	48	39	83	9	19
Stoke Red × Ellis Bitter	1952	40	36	90	13	32
Stoke Red × Backwell Red	1952	40	30	75	16	40
Stoke Red × Frederick	1952	40	33	83	21	53
Stoke Red × Kingston Bitter	1952	40	30	75	13	33
Backwell Red Selfed	1952	120	0	0	0	0
Bulmer's Norman Selfed	1950	204	4	2	2	1
Bulmer's Norman Selfed	1952	44	1	2	0	0
Bulmer's Norman × Improved Foxwhelp	1952	40	40	100	38	95
Bulmer's Norman × Reine des Pommes	1952	44	32	73	29	66
Bulmer's Norman × Sweet Alford	1952	40	40	100	38	95
Bulmer's Norman × Tardive Forestier	1952	44	16	36	12	27
Bulmer's Norman × Tremlett's Bitter	1952	84	61	73	49	58
Court Royal Selfed	1950	228	3	1	0	0
Sweet Alford Selfed	1949	268	1	0	1	0
Sweet Alford Selfed	1950	220	3	1	2	1
Sweet Alford Selfed	1951	168	4	2	1	1
Sweet Alford Selfed	1952	40	0	0	0	0
Sweet Alford × Bulmer's Norman	1952	40	0	0	0	0
Sweet Alford × Reine des Pommes	1952	40	21	52	7	18
Sweet Alford × Tardive Forestier	1952	40	24	60	11	28
Sweet Alford × Tremlett's Bitter	1952	40	27	68	8	20
Yarlington Mill Selfed	1950	216	0	0	0	0

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SOIL CONDITIONERS:

I. THE EFFECT OF THE SODIUM SALT OF HYDROLYSED POLYACRYLONITRILE (CRD189) AND OF CRD186 ON NUTRIENT AVAILABILITY AND UPTAKE BY PLANTS.

By C. BOULD and J. TOLHURST.

Introduction.

For many years soil scientists have studied the effects of organic matter on soil structure and many attempts have been made to determine the nature of the organic constituents responsible for soil aggregation. In recent years (5) it has been demonstrated that certain metabolic products of soil micro-organisms have a beneficial effect on soil structure. These metabolic products, which are mainly polyuronides and polysaccharides, are themselves decomposed by other micro-organisms so that their effect on soil structure is not permanent. Knowledge of the mechanism of action of these naturally occurring substances that improve soil structure led to a search for synthetic organic compounds with similar mechanism but with a relatively slow rate of decomposition in the soil. This search was carried out by the Monsanto Chemicals scientific staff and eventually two water-soluble, polymeric electrolytes, of high molecular weight, were chosen for further investigation because they were effective in extremely low concentrations. These two substances known as CRD189, the sodium salt of a hydrolysed polyacrylonitrile, and CRD186, a vinyl acetate maleic acid co-polymer, which has a higher tolerance for calcium, have been tested in the U.S.A., but only CRD189 was available in this country in any quantity during 1952.

The effects of these two substances on soil structure and crop response have been described by Heydrick and Mowry (2), Allison (1), Martin *et al.* (4) and Weeks and Colter (7). The mechanism of clay aggregation by polyelectrolytes has been described by Ruehrwein and Ward (6).

These two polyelectrolytes dissociate in water to give polyanions with large negative charges. Because of these negative charges it was thought that they might compete with plants for nutrient elements carrying positive charges and so render them less available to crops.

The object of the experiments described in this article was therefore to determine whether CRD189 and CRD186 had any adverse effect on nutrient availability to plants.

Experimental.

All the experiments were carried out on a close-textured loam, containing a high percentage of fine sand and silt, from the Research Station Plot 17. Analytical details of this soil are given in Table I.

1. *Effect of CRD*189 *on exchangeable potassium and magnesium.*

200 g. samples of air-dry soil (2 mm. mesh) were mixed with varying amounts of soil conditioner on a dry-weight basis, and sprayed with 40 ml.

TABLE I.

Analysis of Soil used in Laboratory and Pot Experiments.

	As % air-dry soil
Moisture	1·6
Coarse sand ..	7·8
Fine sand	46·1
Silt	23·5
Clay	17·9
Organic carbon ..	1·7
pH	6·3

of distilled water. After a thorough mixing they were allowed to air-dry. 25 g. sub-samples were taken, in triplicate, and leached with 500 ml. of neutral normal ammonium acetate. The leachates were made up to volume and analysed for potassium and magnesium. The results are shown in Table II.

TABLE II.

Effect of CRD189 on Exchangeable Potassium and Magnesium.
Mean of Three Determinations.

Treatment	As % air-dry soil	
	Exchangeable K	Exchangeable Mg
No conditioner	0·0355	0·0103
Soil + 0·05% conditioner ..	0·0360	0·0104
„ + 0·10% „ ..	0·0341	0·0104
„ + 0·20% „ ..	0·0344	0·0104

The soil conditioner had no significant effect on exchangeable potassium and magnesium.

2. *The effect of CRD*189 *and CRD*186 *on available soil potassium and phosphate as determined by the Neubauer seedling technique.*

100 g. samples of air-dry soil (2 mm. mesh) were treated with varying amounts of the two soil conditioners, mixed with high quality quartz sand and transferred to Pyrex-glass dishes. One hundred rye seeds were placed in each dish, covered with sand, watered and incubated for 17 days at 20–21° C. At the end of this period the roots were washed free from sand and soil, first with tap water and then with distilled water. The plants from each dish were then transferred to beakers, dried at 100° C. and weighed. Organic matter was then oxidised with mixed nitric and perchloric acids and the

digests, after hydrolysis, were filtered and made up to volume. Aliquots were taken for the determination of total potassium and phosphate. All treatments were in quadruplicate.

The effect of treatments on total dry matter, total potassium and phosphate is shown in Table III.

TABLE III.
THE EFFECT OF CRD186 AND CRD189 ON TOTAL DRY MATTER, TOTAL POTASSIUM AND TOTAL PHOSPHATE OF RYE SEEDLINGS. NEUBAUER METHOD. MEAN OF FOUR TREATMENTS.

Treatment	Total K_2O (mg.)	Total P_2O_5 (mg.)	Dry Matter (g.)
Sand only	15·07	10·70	0·88
Sand + soil	41·60	22·80	1·02
,, ,, + 0·05% CRD189 ..	43·70	24·77*	1·06
,, ,, + 0·10% ,, ..	41·72	24·87*	1·06
,, ,, + 0·20% ,, ..	43·65	25·95**	1·02
,, ,, + 0·05% CRD186 ..	46·77*	26·42**	0·96
,, ,, + 0·10% ,, ..	44·62	23·62	0·96
,, ,, + 0·20% ,, ..	42·90	24·10	1·00
S.E. Mean	1·422	0·633	0·033
Sig. diff. $P = 0{\cdot}05$ = *	4·15	1·85	0·096
Sig. diff. $P = 0{\cdot}01$ = **	5·62	2·50	—

Soil conditioners had no significant effect on total dry matter. CRD189 had a significant positive effect on phosphate uptake, the effect increasing with rate of application. CRD186 had a significant effect on phosphate uptake at the lowest level of application only. CRD189 had no significant effect on uptake of potassium. CRD186 had a significant positive effect on potassium at the lowest rate of application only.

The difference between CRD189 and CRD186 on phosphate uptake may be due to a cation effect. It has been shown by Lewis *et al.* (3) that sodium salts increase and calcium salts decrease the availability and uptake of both fertilizer and soil phosphate.

3. *Pot experiment. The effect of CRD*189 *on the uptake of phosphate and potassium by rye plants in the presence and absence of applied P and K.*

900 g. of air-dry soil ($\frac{1}{4}''$ mesh) were weighed into 5″ bitumened clay pots. Soil conditioner and fertilizers were applied to the soil on an air-dry basis and thoroughly mixed with the soil prior to seeding and watering. Phosphate was applied at the rate of 1 g. of NaH_2PO_4 per pot and potassium at the rate of 1 g. of K_2SO_4 per pot. Soil conditioner was applied at two rates, 0·05% and 0·1%. All treatments were in quadruplicate and the pots were arranged in four randomised blocks in a glass-house. Twelve rye seeds, *var.* King 2, after treatment with "Harvesan," were sown per pot. Each pot was then given 100 ml. of a magnesium nitrate solution containing 1 g. of $Mg(NO_3)_2$, followed by routine watering. Seeds were sown on April 4th, 1952. On May 19th the plants became nitrogen deficient and each pot was given a solution containing 1·5 g. of ammonium sulphate. Growth then became vigorous. No visual differences due to treatments could be observed.

The plants were harvested on June 19th, 1952, and separated into "roots" and "tops." The roots were so matted that it was impossible to separate them completely from soil. When dry they were digested with mixed nitric and perchloric acids, and, after filtering, washing and drying, the residual

TABLE IV.

EFFECT OF CRD189 ON DRY MATTER OF ROOTS AND TOPS AND ON TOTAL P_2O_5 AND K_2O OF TOPS ONLY OF CEREAL RYE PLANTS. POT EXPERIMENT.

(a) Dry Matter, Roots (g.)

Fertilizer	Mean of 4 replicates			Mean of 12 replicates	S.E. Mean 12	Sig. dif. $P = 0{\cdot}05$
	Soil only	Soil + 0·05%	Soil + 0·10%			
Nil	8·50	8·10	7·22	10·59	0·424	N.S.
+ P	7·72	6·80	7·50	9·79		
+ K	7·85	6·90	8·05	10·13		
Mean of 12	10·70	9·69	10·12			

(b) Dry Matter, Tops (g.)

Fertilizer	Mean of 4 replicates			Mean of 12 replicates	S.E. Mean 12	Sig. dif. $P = 0{\cdot}05$
	Soil only	Soil + 0·05%	Soil + 0·10%			
Nil	12·27	10·95	10·97	11·39	0·472	N.S.
+ P	9·75	10·78	9·90	10·14		
+ K	10·87	9·78	10·00	10·22		
Mean of 12	10·96	10·50	10·29			

(c) Total P_2O_5 Tops (mg.)

Fertilizer	Mean of 4 replicates			Mean of 12 replicates	S.E. Mean 12	Sig. dif. $P = 0·05$
	Soil only	Soil + 0·05%	Soil + 0·10%			
Nil	122·5	105·3	113·9	113·9		
+ P	137·9	161·4	142·6	147·6*	6·98	20·4
+ K	104·3	103·5	100·4	102·7		
Mean of 12	121·6	123·4	118·9			

* Sig. for $P = 0.05$

(d) Total K_2O Tops (mg.).

Fertilizer	Mean of 4 replicates			Mean of 12 replicates	S.E. Mean 12	Sig. dif. $P = 0·05$
	Soil only	Soil + 0·05%	Soil + 0·10%			
Nil	761·5	695·5	716·2	724·4		
+ P	633·7	741·2	686·7	687·2	31·6	N.S.
+ K	801·7	761·0	716·2	759·6		
Mean of 12	732·3	732·6	706·4			

soil and sand from the digests was weighed. These weights were deducted from the total dry weights of roots and soil. The amount of residual soil was so large and variable that it was felt unjustifiable to determine P and K in the root digests. Total P and K were therefore determined in the " tops " only.

The results for dry matter, phosphate and potassium are given in Table IV (a)......(d).

CRD189 had no significant over-all effect on dry matter of roots or tops of rye plants, or on the uptake of P and K by these plants in the presence and absence of P and K applied as fertilizers.

Discussion.

The results of these experiments, although limited in scope, indicate that a soil conditioner such as the sodium salt of polyacrylic acid, when used in the quantities necessary to improve soil structure, is unlikely to have any adverse effects on the availability or uptake of the major plant nutrients. On the contrary the evidence suggests that under certain circumstances the soil conditioner may increase the uptake of phosphate. This may be due to a cation effect as shown by Lewis *et al.* (3) or to the poly-anion replacing exchangeable phosphate. When samples of soil were shaken with increasing amounts of CRD189 in solution, using a soil-solution ratio of 1 : 10, increasing amounts of soil phosphate became water-soluble.

These experiments were not concerned with the effect of CRD189 on trace element availability, although some determinations were made on the uptake of iron by rye-seedlings using the Neubauer method. The first experiment, in which the seedling roots were washed with water only, indicated that soil treated with CRD189 increased the iron content of the plants. The experiment was repeated using a refined washing technique in which the roots were washed first with water, then dilute hydrochloric acid followed by glass-distilled water. On analysis these plants showed no significant differences in iron content due to the CRD189 treatments. It appears therefore that the increase in iron content in the first experiment was probably due to adsorption of iron on the external root surfaces.

It is hoped to continue these experiments using CRD186 and to study the effect of soil conditioners on the growth and nutrition of strawberries under field conditions. A field experiment with CRD189 was started in 1952, using the variety Climax as the test crop.

Summary.

(1) The sodium salt of hydrolysed polyacrylonitrile (CRD189) had no effect on exchangeable potassium or exchangeable magnesium of a soil when used over the range 0·05 to 0·2% on a dry weight basis.

(2) CRD189 had no effect on available soil potassium but increased available soil phosphate as determined by the Neubauer rye seedling method. The effect on soil phosphate increased with rate of application.

(3) CRD189 had no significant over-all effect on the uptake of potassium and phosphate from soil by rye seedlings grown in pots in the presence and absence of added K and P fertilizers.

Acknowledgment.

The soil conditioners were supplied by Monsanto Chemicals Ltd., to whom thanks are due for their co-operation.

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NUTRIENT PLACEMENT IN RELATION TO FRUIT TREE NUTRITION:

IV. EXPERIMENTS WITH UREA SPRAYS ON FOLIAGE, 1952.

By **J. TOLHURST** and **C. BOULD.**

Since our last report (1) on the use of urea as a foliage spray for apples, a number of American papers have been published on this subject. Fisher (3), as a result of experiments extending over a number of years, has reached the following conclusions regarding the use of urea foliage sprays on apples :

(a) Yields from trees receiving foliage sprays of urea are at least as good as yields from trees receiving soil applications of comparable amounts of nitrogen.

(b) Within the period from the pre-blossom stage to the time of the second cover spray, the later a spray is applied the greater is its effect on leaf nitrogen.

(c) Leaf sprays of urea cause more rapid leaf nitrogen effects than soil applications ; the effects are as great as or greater than those from soil applications of a comparable amount of nitrogen but they are more transient.

Cook and Boynton (2) made a study of the factors affecting the absorption of urea by apple leaves. They found that the lower leaf surfaces absorbed urea at a greater rate than did the upper ones, and that young leaves showed greater uptake than older ones on the same tree. High absorption seemed to be associated with high original leaf nitrogen level, low temperature, and low vapour pressure, while the inclusion of a wetting agent more than doubled the amount absorbed.

More urea was absorbed when the pH of the spray solution was at 5·6 than at pH 8·0. Absorption was most rapid during the first few hours but continued at a measurable rate for at least 48 hours.

Rodney (4) showed that nitrogen as urea, calcium nitrate or ammonium sulphate could enter directly through the leaf cuticle.

Our own experiments were continued at Ottery St. Mary, Centre I, and a new experiment was started at Long Ashton, Centre II. An account of these two experiments follows.

Centre I : Ottery St. Mary.

The layout and treatments were the same as described in the previous report (1). Again, no manures were applied to the experimental area. Management of the grass-plus clover sward was altered, in that a Hayter cutter took the place of a gang-mower, resulting in less frequent cutting and a consequent ageing of the herbage.

A 1% urea spray, with Limpsopon* as wetting agent, was applied on three occasions and in the following quantities :—

	Date	Volume per tree
1st spray	May 22nd (Petal fall)	2·0 gallons
2nd spray	June 5th (Fruitlet)	2·0 ,,
3rd spray	June 20th	2·2 ,,

As in the two previous seasons no spray damage to the leaves was observed at any stage.

Since it was desired to find if there were any residual effects from the treatments of the previous season, leaf samples were taken immediately before the first spray was put on. Similarly, samples were taken before the second and third sprays, and on two later dates, July 24th and September 17th.

Results.

Analyses for total nitrogen showed that there was considerably less variability for trees within each treatment than was noted in 1951. The vigorous growth of clover had apparently maintained the nitrogen supply to the trees at a high level, as in 1951, regardless of treatment. It is known that broadcasting nitrogen fertilisers on a sward containing clover can often prove fatal to the clover (5), and it had been hoped that foliage spraying of urea in orchards would obviate this danger, since the amount of urea in the solution falling on to the sward is small. After three years of such treatments at this centre the vigorous stand of clover in the experimental area indicates that urea sprays have had no deleterious effect.

The high nitrogen status of the trees accounted for the absence of observable differences in leaf and fruit colour in 1952. Favourable climatic conditions enabled fruit colour and quality to be excellent in spite of the high nitrogen level. Yields were high throughout the plot and there was no significant correlation between individual tree yield and leaf nitrogen status, in contrast to that found in 1950.

Table I shows the analytical results for 1952, and for comparison the figures obtained at the end of the 1951 season. In spite of the highly significant differences in 1951, the pre-treatment samples of 1952 are notable for their uniform nitrogen contents, indicating that there was no residual effect as measured by the criterion of total nitrogen in the leaf. Data for overall tree growth have not been obtained. Significant differences at the 5% level of probability are, however, shown at the fourth sampling between the three-spray treatment and the one-spray and control treatments.

This marked influence of the third spray in arresting the seasonal fall in leaf nitrogen was not shown by the 1951 data. The time of application probably corresponds to that of the American "first cover spray," which Fisher (3) records as giving greater response than sprays at earlier stages of development of the leaf. A diagrammatic representation of the relative rates

(*Limpsopon is a solid, related to the sulphonated higher alcohols, and was used at 8 oz. per 100 gallons of spray).

of decrease in leaf nitrogen throughout the season is given in Fig. 1. The treatment means for nitrogen have been adjusted to a common origin and plotted against time. This device shows more clearly the effect of each spray application. Although the analyses of the final sampling of 1952 are in the order expected from the nitrogen treatments, the differences are not significant.

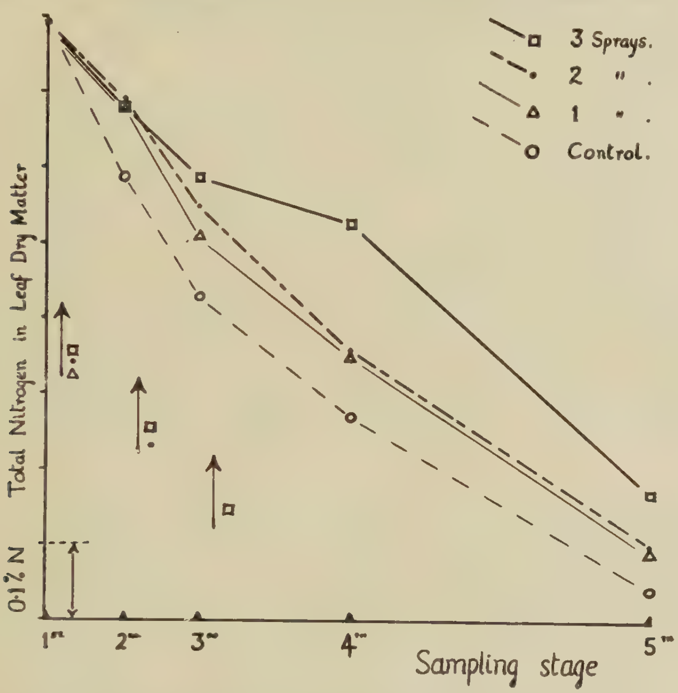

Fig. 1. The relative rates of decrease in leaf nitrogen as influenced by 1% urea foliage sprays on apple, var. Laxton's Superb. Arrows with symbols denote corresponding spray applications.

Centre II : Long Ashton.

A further experiment was started in 1952 to gain more detailed information on the changes in leaf nitrogen induced by spraying apple trees with urea at three different stages of leaf development. The stages chosen were : pink bud, petal fall and fruitlet ; this last period being taken as two weeks after petal fall. Routine spray periods were taken as guides because it was also desired to test the effects of mixing urea with the routine sprays, in the interests of economy in commercial practice.

Site and Treatments. The trees, var. Cox's O.P. on M.II, were planted as maidens in 1947-48 at 4 ft. intervals in rows 8 ft. apart and were trained as dwarf pyramids. Every fourth tree was of the variety Winston, allowing buffered three-tree units of Cox to be used as single plots.

The whole area had been sown with S.48 timothy in 1951 and this had reduced the nitrogen supply to the trees to a very low level, retarding their growth severely. A basal dressing of 3 cwt. per acre of nitro-chalk had been applied to the area in January, 1952, and in addition the following treatments were given :—

1. Control.

2. Nitro-chalk broadcast on the sward at the rate of 3 cwt. per acre on April 4th, 1952.
3. Pink bud foliage spray of 0·5% urea ; April 28th.
4. Petal fall spray of 1% urea ; May 16th.
5. Fruitlet stage spray of 0·5% urea ; June 4th.
6. Sprayed at pink bud and petal fall ; as above.
7. Sprayed at petal fall and fruitlet ; as above.
8. Sprayed at all three stages ; as above.

Limpsopon was used in all sprays as a wetting agent, and a hand sprayer employed. Only the petal fall spray was combined with the routine orchard spray.

Leaf samples from non-fruiting spurs were taken on June 13th, July 23rd and September 18th. The first date was the earliest opportunity of obtaining sufficient material from each plot of three small trees. The second sampling was done when differences in foliage colour had become very noticeable between certain of the treatments.

Design. A randomised block layout, with threefold replication, made full use of the available trees and also permitted the wide variability in experimental material to be distributed between the blocks. This variability was readily shown by tree vigour before the experiment started, and was due partly to a soil fertility gradient and partly to the positioning of the trees in short rows.

Results.

Observation of leaf colour suggested that the ground application of nitro-chalk had stimulated the grass alone, and that the trees had received no benefit ; nor did they benefit from the spray of 0·5% urea at pink bud, at which stage the total leaf area was very small. The fruitlet stage spray improved leaf colour only slightly, but those trees sprayed at petal fall with urea maintained a dark leaf colour for a large part of the season.

Leaf damage resulting from certain treatments is of interest. At pink bud, 0·5% urea was applied alone and a slight burning of the leaf tips occurred ; this appeared to have no adverse effect on the trees subsequently. Combination with lime-sulphur had been avoided because damage from urea alone had been expected on such young leaves.

At petal fall, however, 1% urea was applied in a spray containing $\frac{3}{4}$% lime-sulphur, 2% epsom salts and nicotine, and severe damage resulted. Leaf tips and margins were deeply scorched and often distorted. The variety Winston was only slightly damaged. This spray mixture, but omitting the urea, was put on to the remaining plots in the experiment on the same day and no damage occurred. At harvest the fruits showed no signs of damage, regardless of the treatment. At the fruitlet stage 0·5% urea alone caused no leaf damage.

The weather during the petal fall spraying was very hot, but previous experience with nutrient foliage sprays has not indicated that high temperatures favour leaf damage. A similar combined spray, including urea, had been applied to Cox at another centre and at a comparable time with no ill effects. The experimental area at Centre II adjoined an apple orchard of several varieties which, in spite of their vigorous and healthy growth, suffered badly from the same petal fall spray.

Preliminary tests with the variety Lord Lambourne, again during hot weather, suggested that the total concentration of solutes in the experimental spray had been too high. In another experiment, on Conference pears, sprays of 1% urea plus spreader in 1950 and 1951 caused no damage, while the same treatment on two different occasions in 1952 caused very severe leaf scorching and distortion.

These results suggest that some risk is attached to the use of urea in foliage sprays, and that the factors conducive to leaf damage form a complex problem.

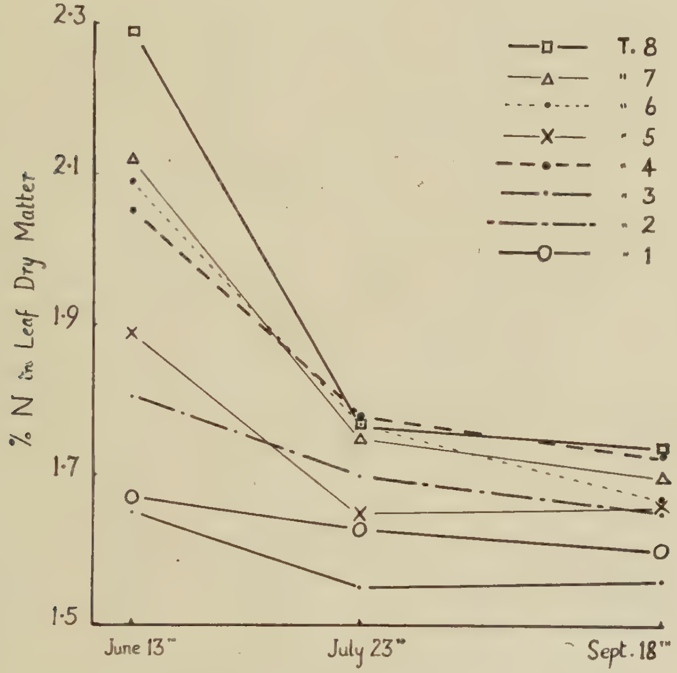

Fig. 2. The effect of urea foliage sprays on the nitrogen content of apple leaves, var. Cox O.P.

T.1.—Control.	T.5.—Fruitlet spray (F.).
T.2.—Ground application.	T.6.—P.B. + P.F.
T.3.—Pink bud spray (P.B.).	T.7.—P.F. + F.
T.4.—Petal fall spray (P.F.).	T.8.—P.B. + P.F. + F.

Analytical results for leaf nitrogen at Centre II are given in Table II, together with tests of significance. Fig. 2 shows these results graphically. The visual observations mentioned earlier in this section are confirmed, although the increase in leaf nitrogen due to the fruitlet stage spray was not significant. The effect of petal fall spraying was highly significant at the first sampling, one month after treatment, but the increase in nitrogen rapidly declined. Pink bud spraying gave no benefit, and the ground application had no significant effect on leaf nitrogen but increased the growth of the grass. The latter effect, by increasing competition for soil water, could constitute a serious problem in orcharding practice. This experiment has indicated the efficiency of urea foliage sprays in supplying nitrogen to the tree without stimulating cover crop growth.

TABLE I.

CENTRE I. EFFECT OF UREA FOLIAGE SPRAYS ON THE NITROGEN CONTENT OF LEAVES : APPLE, VAR. LAXTON'S SUPERB. 1951 TO 1952.

Sampling Date	Mean total nitrogen as % leaf dry matter				S.E. Mean	L.S.D. $P = 0·05$	L.S.D. $P = 0·01$	Remarks
	T1–Control	*ST2	*ST3	*ST4				
2/8/51 . .	1·93	1·98	2·08	2·13	0·036	0·109	0·150	P=0·01 T4>T2, T1 T3>T1
+ 22/5/52 . .	2·69	2·62	2·69	2·63	0·063	0·189	—	N.S.
5/6/52 . .	2·49	2·51	2·54	2·52	0·062	0·187	—	N.S.
20/6/52 . .	2·33	2·34	2·45	2·43	0·054	0·163	—	N.S.
24/7/52 . .	2·17	2·18	2·26	2·37	0·040	0·119	—	P=0·05 T4>T2, T1
17/9/52 . .	1·94	1·92	2·00	2·01	0·042	0·127	—	N.S.

L.S.D. = Least difference between means for significance.
*ST2 = Trees sprayed once, May 22nd.
*ST3 = Trees sprayed twice, May 22nd and June 5th.
*ST4 = Trees sprayed three times, May 22nd, June 5th and June 20th.
+ Figures between the double lines are from leaves sampled in 1952 before the treatments started.

TABLE II.

CENTRE II. EFFECT OF UREA FOLIAGE SPRAYS ON THE NITROGEN CONTENT OF LEAVES: APPLE, VAR. COX O.P. 1952.

Sampling date	Mean total nitrogen as % leaf dry matter								S.E.	L.S.D.	L.S.D.
	T1 Control	T2 Soil application	T3 Pink bud spray (P.B.)	T4 Petal fall spray (P.F.)	T5 Fruitlet spray (F.)	T6 P.B. +P.F.	T7 P.F. +F.	T8 P.B. +P.F. +F.	Mean	$P = 0{\cdot}05$	$P = 0{\cdot}01$
June 13th ..	1·67	1·81	1·65	2·05	1·89	2·09	2·12	2·29	0·0919	0·277	0·387
July 23rd ..	1·63	1·70	1·55	1·78	1·65	1·77	1·75	1·77	0·0733	N.S.	N.S.
Sept. 18th ..	1·60	1·65	1·56	1·73	1·66	1·67	1·70	1·74	0·0817	N.S.	N.S.

Conclusions.

1. Experiments with urea foliage sprays on apples were continued, at Centre I, and extended, at Centre II, in 1952. Leaf nitrogen analyses from Centre I show that there was no residual effect from the preceding year's treatments.
2. Response of leaf nitrogen content to urea sprays was large and rapid, but the maintenance of the benefit would require further applications.
3. The effectiveness of spraying at different stages of development of the leaf is shown diagrammatically. Results at Centre I show the superiority of the late spray and so conform to findings of American workers, but are at variance with the authors' general experience, which suggests that the petal fall spray is the most effective.
4. Urea foliage sprays provide a means of supplying nitrogen to the apple tree with the minimum effect on an orchard cover crop.
5. Leaf damage resulting from the use of urea under varying conditions is discussed, and the problem is shown to be complex.
6. From these experiments it is concluded that urea foliage spraying may prove valuable in practice where conditions require a more precise control over the supply of nitrogen to the tree. The response to such sprays has been remarkably independent of very varied weather conditions.

Acknowledgment.

We are very grateful to Mr. E. N. Pinks, Ottery St. Mary, for his continued help in granting us facilities for our experiment.

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COVER CROPS IN RELATION TO SOIL FERTILITY AND FRUIT TREE NUTRITION: AN EXPERIMENT WITH BUSH CIDER TREES. FINAL REPORT.

By C. BOULD, J. A. H. TOLHURST and R. M. JARRETT.

Previous reports have dealt with the layout, management and aims of this experiment (1), and with the chemical status of the soil and leaves together with some information on symptoms, crop yield and girth increase (2). This report summarises the effect of treatments on soil fertility, girth increase, crop yield and nutrient status of the leaves over the period 1946-51.

Experimental.

The fertiliser treatments were the same throughout with the exception that in the spring of 1951 the plots were split, one half receiving a double dressing of nitrogen.

The composition of the cover crops changed considerably with time, as noted by Luckwill (3). The clover (S.100) soon died out and was replaced by indigenous grasses, mainly *Agrostis alba* var. *stolonifera*. In the spring of 1949 these clover plots were ploughed and re-seeded with the Cotswold strain of wild white clover, at the rate of 4 lb. per acre. Germination was good but owing to poor weather conditions the seedlings made little growth and were soon smothered by weeds. Establishment of clover was poor and by the following year the dominant cover was grass.

Results.

Soil fertility.

Soil samples were taken in August 1951, on a plot basis at 0–6″, 6–12″ and 12″+, and analysed for organic carbon, exchangeable potassium and magnesium and available phosphate. The results are given in Table I.

Comparison of these results with the analysis of soils at the beginning of the experiment (2) shows that the treatments have not had very much effect on the available nutrients.

Clean cultivation caused a substantial loss of organic matter (as measured by the organic carbon) whilst the clover + grass treatment increased the organic matter in the top 12″. However, when corrections were made for initial variations in organic matter content, by analysis of co-variance, these differences just failed to reach significance at the 5% level of probability by analysis of variance of the adjusted values.

Girth increase.

Girth increase of the main trunk was taken as an index of growth. Table III shows the effect of cover crops and rootstock on the girth increase over the period 1946-50. For statistical reasons the logarithm of the girth increase was used in the analysis of variance.

Treatments had no significant effect on girth increase but for all three varieties trees on Crab made more growth than trees on Malling I.

TABLE I.

ANALYSIS OF SOILS (1951) TOGETHER WITH GAINS OR LOSSES OF ORGANIC CARBON OVER THE PERIOD 1946–51.

Plot and depth	Treatment	As % air dry soil—1951				% Gain or loss
		Organic carbon	Ex. K	Ex. Mg.	1% Citric sol. P_2O_5	Organic C
S.1						
0·6″	Annual cover crops	2·31	·020	·011	·028	— 11·9
6–12″		1·01	·011	·008	·004	— 38·4
12″ +		0·81	·008	·008	·003	
S.2						
0·6″	Clean cultivation	2·59	·016	·007	·030	— 10·4
6–12″		0·97	·005	·005	·003	— 48·6
12″ +		0·80	·008	·007	·003	
S.3						
0–6″	Clover (+ grass)	3·30	·020	·013	·019	+ 10·0
6–12″		1·74	·013	·013	·004	+ 17·6
12″ +		0·86	·010	·008	·002	
S.4						
0–6″	Perennial rye grass	3·30	·021	·013	·021	— 2·4
6–12″		1·50	·013	·011	·004	— 7·4
12″ +		0·82	·013	·015	·003	
S.7						
0–6″	Clover (+ grass)	3·61	·032	·010	·022	+ 10·4
6–12″		1·68	·014	·009	·004	+ 30·2
12″ +		0·93	·013	·013	·002	
S.8						
0–6″	Clean cultivation	2·98	·022	·010	·028	— 13·4
6–12″		1·25	·012	·008	·004	— 9·4
12″ +		0·68	·011	·007	·002	
S.9						
0–6″	Perennial rye grass	3·16	·027	·009	·035	+ 6·4
6–12″		1·58	·016	·008	·009	— 9·7
12″ +		0·53	·011	·006	·010	
S.10						
0–6″	Annual cover crops	3·34	·022	·010	·018	+ 7·4
6–12″		1·94	·013	·008	·010	+ 20·5
12″ +		0·99	·010	·007	·007	

Crop yields.

Crop yields were recorded on a tree basis. In the analysis of the results yields have been grouped in two-yearly periods in order to minimise the effects of biennial bearing.

The results for the period 1946-51 are summarised in Table IV. Treatments had no significant effect on the yield of fruit from Sweet Alford or Stoke Red.

TABLE II

ADJUSTED VALUES FOR ORGANIC CARBON AS % AIR-DRY SOIL.
SAMPLED 13TH AUGUST, 1951.

Depth	A Perennial rye grass	B Clover + grass	C Annual cover crops	D Clean cultivation	S.E. Mean	Sig. diff. $P = 0{\cdot}05$
0 – 6″ ..	3·18	3·42	2·95	2·74	0·197	0·682
6 – 12″ ..	1·49	1·81	1·45	1·08		

There was a significant increase in the yield of fruit from Dabinett due to grass but when corrections were made for initial tree size, by analysis of covariance, the adjusted yields showed no significant differences. With all three varieties the yield from trees on Crab was greater than from trees on Malling I.

Leaf nutrient status.

Leaf samples were taken for chemical analysis, at the end of August each year, from the middle third of current year's growth. The mean values, over the period 1947-51, for N, P_2O_5, K_2O and MgO are summarised in Table V. The detailed comparisons and significant differences between treatments are given in the remarks column of Table V. Generally speaking leaves from trees under grass, or clover plus grass, had a lower nitrogen and magnesium content, and a higher phosphate and potassium content than leaves from trees under annual cover crops or clean cultivation. The effect of treatments on leaf magnesium status was not statistically significant but trees on Crab had a higher magnesium status than trees on Malling I.

The effect of treatments on the nutrient status of leaves from Sweet Alford, over six seasons, is shown in Fig. 1.

From Fig. 1 it will be seen that the grass effect reaches its maximum for potassium in the second season, whereas the maximum effect on nitrogen and phosphate does not occur till the third and fourth seasons respectively.

Fig. 1 also illustrates the inverse relationship between nitrogen status, and potassium and phosphate status. The relationship between the mean values given in Table V for N and K_2O, and N and P_2O_5, is almost linear.

Discussion and Conclusions.

This experiment shows that the nature of the orchard cover crop has a highly significant effect on the nutrient status of the tree, as determined by leaf analysis. The effect on the growth and yield of fruit of an established tree is less marked. One must bear in mind, however, that these trees had been growing under conditions of clean cultivation for a period of twelve years before the cover crop experiment began. During that period their framework was formed and the nitrogen status of the leaves at the start of the experiment was high. Grassing down with perennial rye grass reduced the

TABLE III.

EFFECT OF COVER CROPS AND ROOTSTOCK ON GIRTH INCREASE, 1946–50.
(MEASUREMENTS CONVERTED TO LOGARITHMS).

Variety	A Perennial rye grass	B White clover	C Annual cover crops	D Clean cultivation	Sig. Diff.	Rootstock		Sig. Diff.		General	Remarks
					* $P=0{\cdot}05$	Crab	M.I.	** $P=0{\cdot}01$	* $P=0{\cdot}05$	Mean	
Sweet Alford ..	1·0909	1·1431	1·0991	1·1139	N.S.	1·1797	1·0438	0·0790	—	1·1117	**Crab > M.I.
Stoke Red ...	1·1333	1·1553	1·1504	1·0590	N.S.	1·1666	1·0824	0·0098	—	1·1245	**Crab > M.I.
Dabinett ..	0·8055	0·8575	0·7215	0·7638	N.S.	0·8463	0·7279	N.S.	0·0791	0·7871	* Crab > M.I.

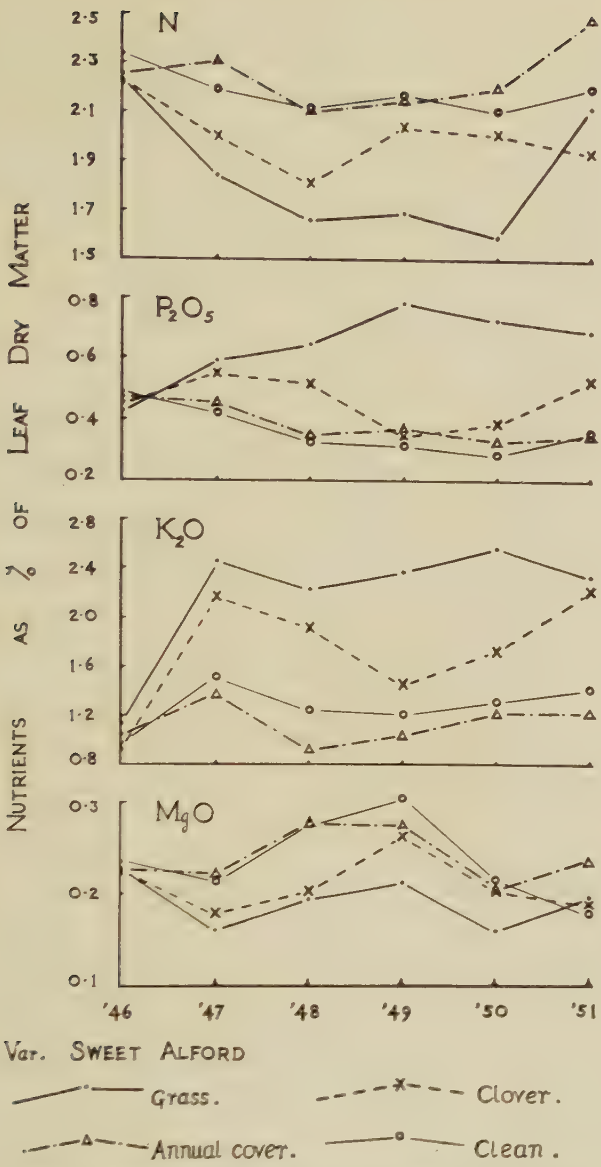

Fig. 1. Showing the effect of cover crops on the seasonal variation in nutrient status of apple leaves, *var.* Sweet Alford.

leaf nitrogen status to deficiency level during the first three seasons, despite the application of 3 cwt. of nitrochalk per acre. This decrease in nitrogen was accompanied by a rapid increase in the potassium status of the leaf, from deficiency level at the onset to luxury consumption in two seasons, and by a less rapid but marked increase in the phosphate status of the leaf. During this period the composition of the sward changed considerably, the rye grass being largely replaced by *Agrostis alba, var. stolonifera.* The changes in the

TABLE IV.

EFFECT OF COVER CROPS AND ROOTSTOCK ON 2-YEAR YIELDS. MEAN VALUES IN POUNDS PER TREE.

Variety. 2 Year Period	A Perennial rye grass	B White clover (+grass)	C Annual cover crops	D Clean cultivation	Sig. Diff.	Rootstock		Sig. Diff.		General mean	Remarks
					* $P=0{\cdot}05$	Crab	M.I.	** $P=0{\cdot}01$	* $P=0{\cdot}05$		
SWEET ALFORD 1946 + 47 . .	110·0	74·8	71·0	74·6	N.S.	76·1	89·1	—	N.S.	82·6	
1948 + 49 . .	297·3	296·8	295·7	319·0	N.S.	343·8	260·6	—	N.S.	302·2	
1950 + 51 . .	463·1	372·4	401·5	354·5	N.S.	462·6	333·1	N.S.	83·5	397·9	* Crab > M.I.
TOTAL 1946–51 . .	870·4	744·0	768·2	748·1	N.S.	882·5	682·8	N.S.	183·9	782·7	* Crab > M.I.
STOKE RED 1946 + 47 . .	156·2	131·5	235·5	125·8	N.S.	161·7	162·8	—	N.S.	162·2	
1948 + 49 . .	174·2	180·9	278·5	138·3	N.S.	221·9	164·0	N.S.	46·5	193·0	* Crab > M.I.
1950 + 51 . .	335·6	319·5	466·0	237·2	N.S.	378·8	300·4	N.S.	46·5	339·6	* Crab > M.I.
TOTAL 1946–51 . .	666·0	631·9	980·0	501·3	N.S.	762·4	627·2	N.S.	97·9	694·8	* Crab > M.I.
DABINETT 1946 + 47 . .	206·6	142·9	103·7	101·2	74·7†	167·5	109·6	N.S.	42·9	138·6	* Crab > M.I.
1948 + 49 . .	221·0	184·0	114·4	107·0	74·7†	195·6	117·6	71·7	—	156·6	** Crab > M.I.
1950 + 51 . .	272·6	188·7	101·7	118·1	74·7†	214·4	126·3	71·7	—	170·3	** Crab > M.I.
TOTAL 1946–51 . .	700·2	515·6	319·8	326·3	217·5†	577·5	353·5	200·3	—	465·5	** Crab > M.I.

† When corrections are made for initial tree size, by analysis of co-variance, these differences are found to be not significant.

TABLE V.

EFFECT OF COVER CROPS ON THE NUTRIENT STATUS OF APPLE LEAVES. MEAN VALUES FOR 1947–51 AS % LEAF DRY MATTER.

Variety and Nutrient	A Perennial rye grass	B White clover (+ grass)	C Annual cover crops	D Clean cultivation	Sig. Diff.		Rootstock		Sig. Diff.		Remarks
					* $P=0{\cdot}05$	** $P=0{\cdot}01$	Crab	M.I.	* $P=0{\cdot}05$	** $P=0{\cdot}01$	
SWEET ALFORD Total N ..	1·783	1·989	2·251	2·161	0·226	—	2·087	2·005	0·056	—	* C, D > A., C > B. * Crab > M.I
,, P_2O_5 ..	0·689	0·470	0·373	0·346	0·168	—	0·474	0·465	N.S.	N.S.	* A > B, C, D.
,, K_2O ..	2·397	1·900	1·173	1·349	0·404	0·741	1·726	1·684	N.S.	N.S.	** A > C, D. * A > B > C, D.
,, MgO ..	0·179	0·203	0·239	0·234	N.S.	N.S.	0·237	0·191	—	0·033	** Crab > M.I.
STOKE RED Total N ..	1·811	1·903	1·988	2·040	0·112	—	1·959	1·911	0·044	—	* C, D > A., D > B. * Crab > M.I.
,, P_2O_5 ..	0·508	0·404	0·339	0·334	0·048	0·088	0·377	0·416	0·021	—	** A > B, C, D.
,, K_2O ..	2·258	1·788	1·790	1·454	0·390	—	1·838	1·807	N.S.	N.S.	* A > B > C, D. M.I. > Crab * A > B, C, D.
,, MgO ..	0·280	0·314	0·376	0·310	N.S.	N.S.	0·362	0·278	—	0·035	** Crab > M.I.
DABINETT Total N ..	1·770	2·026	2·223	2·230	0·175	0·312	2·075	2·050	N.S.	N.S.	** C, D > A. * C, D > B > A.
,, P_2O_5 ..	0·603	0·471	0·397	0·362	0·080	0·147	0·450	0·466	0·015	—	** A > C, D. * A > B > D. M.I. > Crab
,, K_2O ..	2·264	1·754	1·223	1·480	0·426	0·232	1·692	1·699	N.S.	N.S.	** A > B, C, D., B > C. * A > B > D > C.
,, MgO ..	0·233	0·244	0·256	0·244	N.S.	N.S.	0·269	0·219	—	0·022	** Crab > M.I.

nutrient status of the leaf were brought about by a mixed grass sward and are not due specifically to perennial rye grass. The effect of clover on leaf nutrient status was intermediate between that of grass and clean cultivation. The clover disappeared in three seasons and was replaced by indigenous grasses. In the first season the dominant grass was *Poa annua* but this was in turn replaced by *Agrostis alba.*

In March 1949, the clover plots were broken up by discing and prepared for re-seeding, which took place in April. The effect of this soil treatment is reflected in the nutrient status of the leaves sampled in the following August (see Fig. 1). The nitrogen content of the leaves was raised and the phosphate and potassium contents fell. On the other hand when additional nitrogen was added to all the plots in the spring of 1951 the nitrogen status of the leaves was raised considerably, particularly on the grass plots, without causing a corresponding decrease in the phosphate and potassium contents. The differential effect in the two cases may be due to root disturbance. When the clover ley was broken up nitrogen would be released by decomposition of organic matter. Being water-soluble, and in the nitrate form not adsorbed by the soil colloids, it could be translocated to the deeper feeding tree roots. The available phosphate and potassium, however, is confined mainly to the top 9–12″ of soil. Destruction of the surface feeding roots of the tree by discing would interfere with absorption of P and K to a greater extent than with absorption of N. This might account for the fall in the P and K status of the leaves from clover plots during 1949.

When additional nitrogenous fertiliser was applied to the plots in 1951 there was no surface root disturbance. Increased nitrogen absorption under these circumstances was not accompanied by a significant decrease in the P and K status of the leaf.

It should be pointed out that in this experiment we are dealing with percentages and not with total amounts of nutrients. We cannot say that grass has increased the total uptake of P and K by the tree but only that it has affected the composition of the leaf. This matter is being further investigated by means of pot experiments with particular reference to the effect of grass on the uptake of fertilizer and total phosphate.

Cover crops did not affect to any marked degree the available P, K, Mg status of the soil, as determined at one particular time in 1951. It is not known, however, how the treatments affected the rate of supply of available nutrients throughout the season. Other data, not included in this paper, showed that the soil nitrate level during the growing season varied greatly with treatments, and other workers have shown that the available potassium under grass cover is usually greater than under clean cultivation.

Cover crop treatments affected the organic matter status of the soil, although the values after adjustment just failed to reach significance. The grass plus clover ley was the most effective cover crop for improving soil organic matter.

The annual cover crops had no significant effect on any of the factors under investigation. It is doubtful therefore whether their use in commercial practice can be justified under similar climatic conditions to those prevailing at Long Ashton, with an annual rainfall around 35″.

In conclusion it can be stated that a grass or grass-plus-clover cover, properly managed, has many advantages over clean cultivation. Pruning, spraying and harvesting are facilitated, and soil structure and organic matter status are maintained, or improved. The grass cover influences the nutrient status of the leaf. Additional nitrogen, possibly 2 to 3 cwt. per acre or more, depending on the climatic conditions, is required particularly in the first year or so after grassing down. The data suggest that considerable economy could be made in the use of potassium and phosphate fertilizers on grass orchards.

Summary.

1. The effect of cover crops on soil fertility, tree girth, crop yield and leaf nutrient status over the period 1946-51 are summarised.
2. Grass-plus-clover leys made the greatest contribution to soil organic matter.
3. Cover crops had no significant effect on girth increase or total yield of fruit.
4. Grass increased the potassium and phosphate status of the leaf and decreased the nitrogen status as compared with clean cultivation. Annual cover crops had no significant effect on any of the factors investigated.
5. Girth increase, crop yield and leaf magnesium were greater when trees were grown on Crab than on Malling I rootstock.

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SPRAY APPLICATION PROBLEMS

I. THE DISTRIBUTION OF COPPER AND LIME SULPHUR DEPOSITS.

By J. T. MARTIN.

Introduction.

Considerable progress has been made in recent years in developing chemicals for crop protection purposes. New insecticides, fungicides and weedkillers have been introduced, and much attention has been paid to the formulation and standardization of the materials marketed for use by the growers. As a result, many types of chemical product are now available that may be relied upon for quality and performance. As yet, however, we know little of the basic factors, apart from the content of active ingredient, that determine the efficiency of sprays, and much work is needed on application problems if the materials at our disposal are to be used as effectively and cheaply as possible. The problems involved are many and varied and of great complexity. Important advances undoubtedly would follow a better understanding of spraying methods, but progress will only be achieved by close collaborative work of the biologist, the chemist, the physicist and the engineer.

Renewed interest is being shown in methods of applying spray materials. In attempts to reduce operational costs and to limit wastage, automatic and low-volume methods of application have been used. More recently, application by the "mist-drifting" and "air-flow" technique has come to the fore. With these methods, much work is needed, on a quantitative basis, in assessing the performance of the machines that have been developed and in determining the conditions for the maximum impact of sprays on various types of plant target. The use of mists and of droplets propelled by an air-flow has accentuated the problem of drift, but the precise significance of this factor has yet to be determined.

The effective control of many plant parasites is dependent upon adequate coverage and wetting. Highly efficient wetting agents have been produced and these are now being used in pest control products. Valuable work on wetting agents in crop protection has been done in the past, but the development of the new materials calls for a re-examination of the position. It may well be that for the different types of active ingredient now in use there exist optimum types and concentrations of wetters. While insufficient wetting may lead to ineffective control, too efficient wetting may result in excessive drainage of the toxicant from the treated surface. At present, we have little knowledge of the levels of deposits obtained following the commercial application of sprays or of the persistence of residues under the climatic conditions in this country. Adjuvants such as wetters may influence initial deposits or their retention by plant surfaces, while the nature of the surface treated itself may play a part in determining the effect obtained. Consumer hazards may arise following the use of the more persistent chemicals that are toxic to humans.

The widespread use of insecticide formulations such as dispersible powders raises special considerations. The materials must show adequate suspension in the tank during spraying to avoid the unequal application of the active principle, while the range of particle size of the insecticidal constituent may have an important bearing on the degree of insect control obtained. Methods are required for the examination of the physical properties of spray materials so that these may be correlated with biological performance with a view to improving efficiency. The factors responsible for the often unpredictable phytotoxic effect of some of our spray chemicals are little understood. Apart from the question of compatibility of the ingredients, both active principles and adjuvants, of a mixed wash, the variety and possibly the environmental conditions of the plant treated may determine whether a damaging effect is seen.

Protective fungicides such as copper compounds rely for their action upon some degree of permanence of the deposits. These may rapidly be removed by rainfall, particularly under tropical conditions, and the possibility arises of extending the effectiveness of the materials by the use of suitable adhesives. Suggestions have been made of the redistribution of fungicide deposits over treated surfaces by the action of rain, but we have no detailed knowledge of the extent to which this may take place, or indeed, of the area over which individual particles, deposited on a leaf surface, are able to exert their protective effect.

Special interest in application problems of this kind is now taken by the Plant Pathology sections at Long Ashton, and the results of collaborative work will be reported in subsequent papers in this series. Advances depend, to a large extent, upon the availability of suitable methods for studying spray deposition. The *distribution* of a deposit, rather than the total amount that may be found, by chemical assay, on a given surface, is a factor of major importance in many of our spraying problems. In devising methods for the examination of distribution, we have aimed at tests that may easily be carried out in the field and that provide permanent records of the results obtained. The simple method described here for copper and lime sulphur utilises the transference of the deposit from the perishable plant tissue to a permanent plaque. It may have application for other materials provided suitable colorimetric reactions are available.

Copper Deposits.

Copper compounds give highly coloured salts with organic reagents such as rubeanic acid and sodium diethyldithiocarbamate. The reactions are sensitive and detect copper when present in very small amounts. An assessment of the distribution of a copper fungicide over a leaf may be obtained by bringing the surface into contact with filter paper soaked in a suitable reagent. This method, while useful, has its limitations. Deposits tend to accumulate in depressions and may not be picked up. This difficulty has been overcome by the simple expedient of preparing an impression of the treated surface in plaster of Paris.

Method for copper deposits.

Anhydrous calcium sulphate (20 g.) is creamed with water (about 12 ml.) and run on to a glass plate. As the mixture develops "tackiness" before setting, the surface holding the deposit is gently pressed in, taking care to avoid the occlusion of air. The plaque is allowed to harden for some twenty minutes, and the plant material is then carefully peeled off. The plaque, which retains the deposit, is then wetted with a 0·2 per cent aqueous solution of the dithiocarbamate reagent when the imprint of the distribution of the fungicide develops. The plaques may be kept indefinitely and the results recorded photographically.

The residues from small droplets (0·0002 ml.) of dilute suspensions of copper fungicides (down to 0·01 per cent Cu) placed singly upon smooth and hairy leaf surfaces were recorded satisfactorily. With experience of the method, it is possible that an approximate quantitative assessment of distribution may be made by comparison of the intensity of coloration at different points of the plaque with that from standardised droplets of the same fungicide upon the same plant surface. We have used the method to study the extent and distribution of copper deposits, following different treatments, on bean, potato, tomato and banana leaves. Some examples are given in Fig. 1. The method is likely to prove particularly useful in studying application problems under tropical conditions where loss and redistribution of copper by rainfall are important considerations.

Lime Sulphur Deposits.

The application of the plaque method for recording deposits of lime sulphur has been studied by Mr. G. V. Coles. In developing the technique for this material, the changes taking place in lime sulphur solution on deposition on foliage have been re-examined and the earlier findings, as reviewed by H. Martin (1940) have been confirmed. The yellow colour is rapidly lost, elemental sulphur is deposited and the nitroprusside reaction for sulphides is no longer given.

The absence of sulphides in the dried spray deposit on leaves was further indicated by the failure, in the plaque technique, to obtain colour reactions, under suitable conditions, with salts of bismuth, copper, cobalt and nickel. Silver gave a stain, but the plaque itself gradually became discoloured. The presence of elemental sulphur was confirmed by the thallium test of Feigl (1946) and this was adapted for recording deposits.

Methods for lime sulphur deposits.

(a) *Thallium test.* Anhydrous calcium sulphate (20 g.) is mixed with 1 per cent thallium sulphate solution (12 ml.) and 2·5 per cent sodium sulphide solution (4 ml.) is stirred in, when the cream blackens. The impression of the surface holding the deposit is then made. After setting, the plant material is removed, the plaque is bleached by immersion for a short period in 0·5 per cent nitric acid solution and rinsed in water. The deposits then show as red-brown markings of thallium polysulphide. The method is specific for sulphur and selenium.

(b) *Silver test.* An improved method was devised using silver. The calcium sulphate is creamed with 0·5 per cent silver nitrate and the impression made. Care should be taken to avoid bruising the plant tissue. After setting and removal of the plant material, the plaque is immersed for 30 seconds in a fixing solution of 20 per cent sodium thiosulphate in 1·25 per cent potassium metabisulphite and then well washed in a bath of running water. The plaques may be stored without discoloration if kept in the dark ; some examples are shown in Fig. 2. We have used the test successfully to assess the performance of an " air-flow " machine, and to compare the efficiency of this method of application with conventional lance-spraying of lime sulphur for the control of apple scab.

Summary.

Spray application problems are reviewed, and simple methods are described for recording the distribution of copper and lime sulphur spray deposits on plant surfaces.

Acknowledgment.

I am indebted to Mr. G. H. Jones, A.R.P.S., for the photographs.

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PLATE V

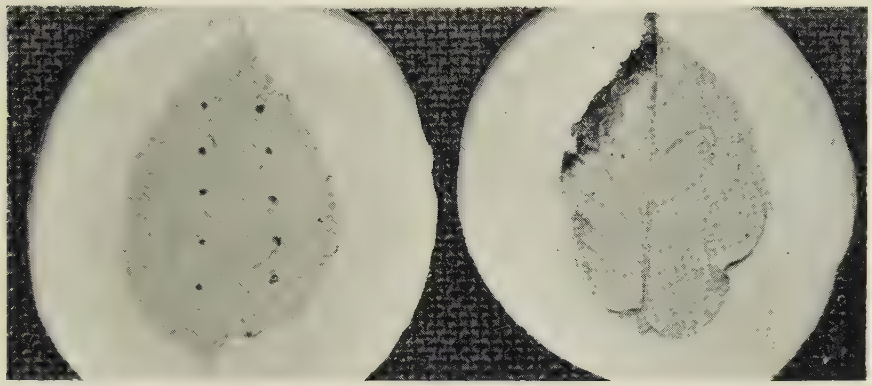
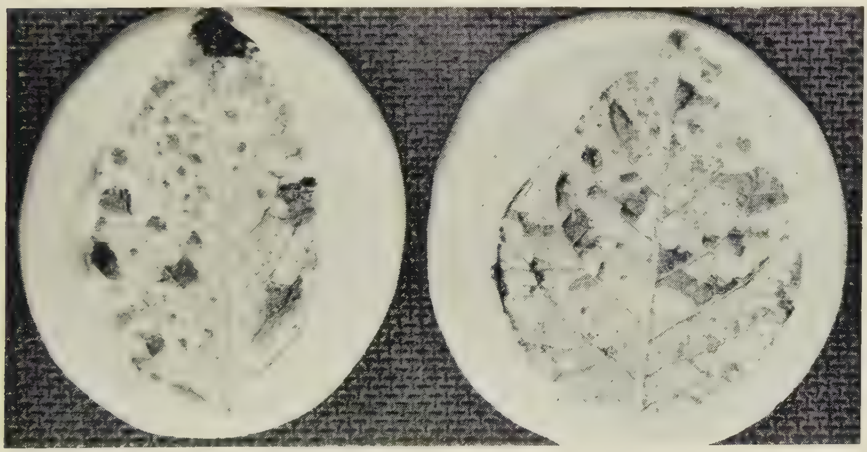


Fig. 1. COPPER SERIES.

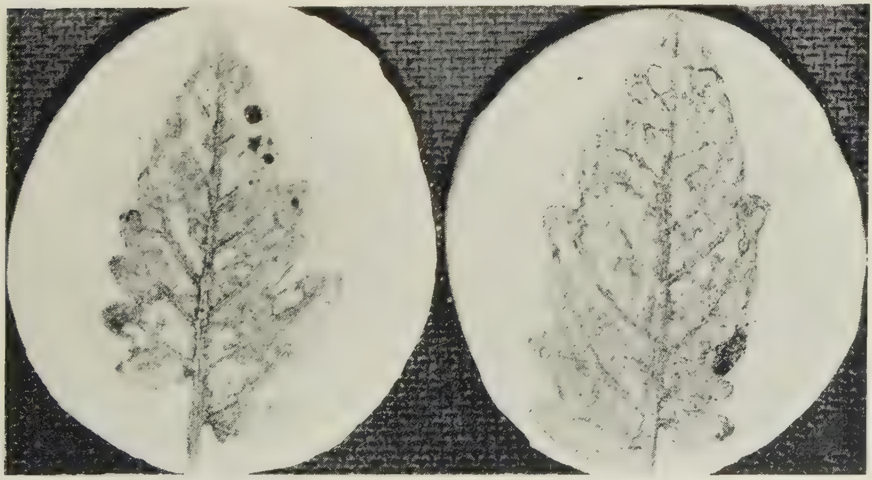
A(a) Placed droplets of copper oxychloride suspension (0·2% Cu) on potato leaf, under surface.

A(b) Distribution of copper oxychloride suspension (0·2% Cu) on potato leaf, upper surface, after spraying to run-off.



B(a) As A(b)

B(b) As B(a), under surface.



C(a) Distribution of Bordeaux Mixture (0·2% Cu) on tomato leaf, under surface, after spraying to run-off.

C(b) As C(a), with wetter added.

PLATE VI

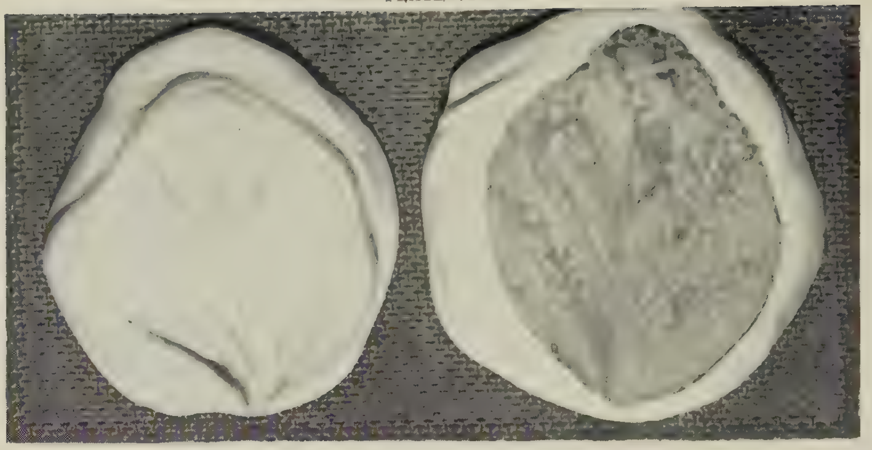
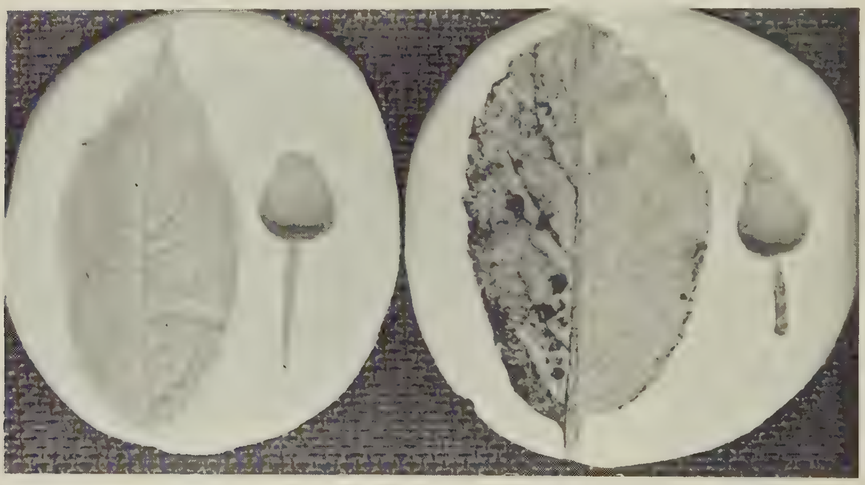


Fig. 2. Lime Sulphur Series.

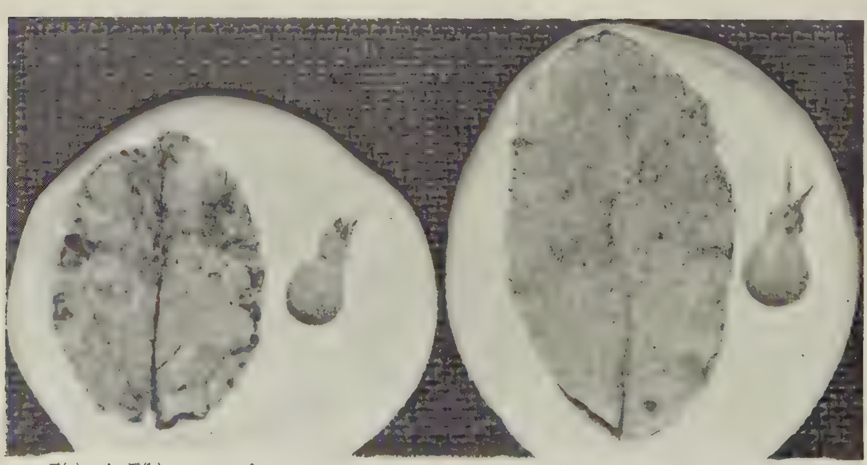
D(a) Bean leaf untreated, under surface.

D(b) Distribution of lime sulphur (1·0%) on bean leaf, under surface, after spraying to run-off.



E(a) Apple leaf, under surface, and fruitlet untreated.

E(b) Distribution of lime sulphur (1·0%) on apple leaf, under surface, and fruitlet after spraying to run-off.



F(a) As E(b), upper surface.

F(b) As F(a), with wetter added.

SPRAY APPLICATION PROBLEMS
II. DDT AND COPPER RESIDUES ON SOFT FRUITS.

By J. T. MARTIN, R. F. BATT and PAULINE JACKSON.

Introduction.

The discovery during the past ten years of insecticides such as DDT, BHC and the organo-phosphorus compounds has enabled us to control with greater ease and certainty many of the important pests of fruit, vegetable and farm crops. Their introduction, however, has accentuated other problems. The chemicals are toxic in varying degrees to man and some show a degree of persistence that may lead to residues on crops at harvest time.

The possibility of cumulative effects on man arising from the ingestion, over long periods, of foodstuffs contaminated by potentially toxic chemicals is causing increasing concern. Steps have already been taken in this country to ensure a measure of safety. A Sub-committee of the Food Standards Committee of the Ministry of Food has recommended maximum permissible limits for copper (Anon., 1951) and lead and arsenic (Anon., 1952) in articles of diet. These recommendations state that copper in general should not exceed 20 parts per million (p.p.m.*), lead 2 p.p.m. and arsenic 1 p.p.m.; modifications of these levels are suggested for some foods and beverages. A maximum limit for DDT of 7 p.p.m. and for gamma BHC of 2·5 p.p.m. (based on the assumption that the content of the gamma isomer in the BHC used was not less than 12 per cent) have been considered in this country to be reasonable. In the U.S.A., a tolerance of the order of 5–7 p.p.m. DDT has been accepted in the past, but more recently there has been a tendency to regard unfavourably any DDT contamination of foodstuffs. No tolerance for parathion has yet been suggested. David and his colleagues (1951) have examined the position with regard to schradan, and from a consideration of the toxicological evidence so far available, suggest that for crops that constitute a minor or seasonable article of diet, or which are diluted before consumption, a limit of 3 p.p.m. should prove satisfactory. A joint Working Party of the Ministries of Food and Agriculture and Fisheries has examined the possible risks to the consumers of crops treated with spray materials and its Report is awaited with interest.

Crop protection methods were surveyed and possible hazards to consumers were examined at a Conference held in 1951 by the Society of Chemical Industry (Martin 1952). While many of the commonly used insecticides, fungicides and weedkillers could be exonerated, a full assessment of the position could not be made because of the lack of information at that time. Since then Timberlake and Pollard at Long Ashton have extended the work of Shaw (1936) and Shaw and Steer (1937, 1938) on lead arsenate and have traced the fate of lead and arsenic on apples in the subsequent processing of the fruit. Their results are given on page 151 of this Report. This preliminary paper records the data obtained, over two seasons, for DDT residues on soft fruits and for copper residues on tomatoes.

*P.p.m. throughout this paper are expressed on fresh weight.

DDT

Although derris is a safe and effective remedy, DDT may be used by growers for the control of the gooseberry sawfly and raspberry beetle. DDT is also employed against the leaf-curling midge of blackcurrant and for the control of tomato moth under glass. Treatment is given when developing fruits are present, with risk of contamination. Trials were therefore made with DDT dispersible powder and emulsion at the strengths normally used, and simulating commercial conditions as closely as possible.

Method of analysis. A colorimetric method was used, based on the formation of a blue colour on treating nitrated DDT with alcoholic alkali, following earlier work by Schechter and his colleagues (1945) and, in this country, by Illing and Stephenson (1946).

Our aim in the method has been to eliminate any production of colour with extracts of untreated crops, and this has been accomplished with foliage and fruits. Carbon tetrachloride was used for the initial extraction in preference to benzene since the latter, unless completely removed, may cause interference after nitration. Plant wax and pigment were largely removed by passing the concentrated initial extract through a column of activated alumina (see also Norton and Schmalzreidt, 1951). The solvent was removed from the percolate and the residue nitrated by heating in a boiling water-bath for some 30 minutes with a mixture of equal parts of fuming nitric and concentrated sulphuric acids. The acid solution was diluted with water, the nitrated DDT extracted with ether and the ether solution washed, to distinct alkalinity, with successive small portions of dilute potash in salt solution. This procedure finally removed any pigment not retained by the alumina or formed during nitration. Excess alkali was removed from the ether solution by washing with salt solution, the ether layer was run through anhydrous sodium sulphate and the solvent removed. The blue colour was then developed, in benzene solution, by the addition of alcoholic potash.

The depth of colour produced was compared visually, or with a Duboscq-type colorimeter, with standards prepared from pure *pp′*-DDT or from extracts of plant material, sprayed with the carrier alone, to which known amounts of *pp′*-DDT were added. Control tests with DDT-free plant extracts were also included. The most satisfactory level for colour comparison was found to be in the range 0·1 to 0·4 mg. DDT.

Under the conditions of the method, *pp′*-DDT and 1,1-bis(4-chlorphenyl)-2,2-dichlorethane (DDD) give a blue colour, *op′*-DDT a rich magenta, 1,1-bis(4-chlorphenyl)-2,2-dichlorethylene (DDE) a bright orange red and 4,4′-dichlor-benzophenone a rich tangerine colour. The last compound, said by Fleck (1949) to be an important oxidation product of DDT under the influence of ultra-violet light, is the one most likely to invalidate the results, but is removed by the alumina.

Application of the method.

Gooseberries. Bushes were sprayed in 1951 with a proprietary DDT dispersible powder, a benzene-sulphite lye-DDT emulsion, both at 0·1 per cent DDT in the spray fluid, or with the benzene-sulphite lye carrier alone. Samples of fruits were taken for analysis immediately after spraying and 15 and 41 days later.

A progressive decline in residues, as p.p.m., was to be expected from the increase in weight as the fruits developed. To determine whether there was an actual loss of DDT by weathering, the residues recorded over the period were also expressed as mg. DDT per hundred fruits. The results are given in Table I.

TABLE I.

DDT RESIDUES ON GOOSEBERRIES 1951.

Date of sampling	Condition of fruits	Average weight of fruit (g.)	Dispersible powder		Emulsion	
			DDT p.p.m.	DDT mg./100	DDT p.p.m.	DDT mg./100
29 May ..	Small, green	1·85	14	2·4	14	2·9
13 June ..	Green	3·94	6	1·9	7	2·7
9 July ..	Ripe	6·10	4	2·1	3	1·8

Over the period of the experiment, 344 hours of sunshine and 0·97 ins. of rain were recorded.

Analysis of the leaves showed that there was a progressive loss in residues due to weathering. Initial DDT deposits of the order of 250 p.p.m. fell to levels of approximately 50 p.p.m. over the period of 41 days.

The test was repeated in 1952 using the benzene-sulphite lye emulsion at 0·1 per cent DDT. The results are given in Table II.

TABLE II.

DDT RESIDUES ON GOOSEBERRIES, 1952.

Date of sampling	Condition of fruits	Average weight of fruit (g.)	DDT p.p.m.	DDT mg./100
13 May	Small, green ..	1·73	29	5·2
23 May	Green	2·98	14	4·3
12 June	Large, green ..	4·80	6	2·8
30 June	Ripening	6·43	3	2·1
23 July	Overripe	6·87	3	1·7

499 hours of sunshine and 4·8 ins. of rain were recorded. The initial deposit on leaves was 350 p.p.m.

Raspberries. A similar test was carried out in 1951 on raspberries. Canes were sprayed with the benzene-sulphite lye emulsion at 0·1 per cent DDT, when the fruits were small and green and on the point of reddening. Because of the difficulty of separating the immature fruits and calyces, the analyses immediately after application and seven days later were carried out on the fruits and calyces as a whole. The final analysis was made on the consumable ripe berries, without receptacles and calyces. The results are given in Table III.

TABLE III.

DDT RESIDUES ON RASPBERRIES, 1951.

Date of sampling	Condition of fruits	Average weight of fruit (g.)	DDT p.p.m.	DDT mg./100
13 July	Green, reddening ..	1·19	43	4·3
20 July	Red	1·2	20	2·3
26 July	Ripe	2·2	8	1·7

The calyces with attached receptacles separated at the time of the third sampling showed 55 p.p.m. DDT.

106 hours of sunshine and 0·56 ins. of rain were recorded during the period of the test.

Loganberries and blackberries. DDT may also be used on these crops for the control of the *Byturus* beetle. Tests were therefore made as before, using the benzene-sulphite lye emulsion at 0·05 per cent DDT. The results obtained with loganberries from which the calyces were removed are given in Table IV.

TABLE IV.

DDT RESIDUES ON LOGANBERRIES, 1952.

Date of sampling	Condition of fruits	Average weight of fruit (g.)	DDT p.p.m.	DDT mg./100
24 June	Large, green, some pink ..	2·1	17	3·6
21 July	Ripe	4·3	2	0·9
24 July	Overripe	4·4	1	0·5

216 hours of sunshine and 0·66 ins. of rain were recorded during the test.

The calyces alone showed a progressive decline in DDT residues, as p.p.m., from 230 to 150.

Blackberries. The final DDT deposit on consumable cultivated blackberries was of the order of 2 p.p.m.

Blackcurrants. For the control of the leaf-curling midge, DDT should be applied as soon as the pest is seen, but this may not be until late in the period of fruit development. Trials were therefore made with the application of DDT at an early stage in fruit formation and also at a later stage on previously unsprayed bushes when fully formed fruits were present. The DDT was applied, as the benzene-sulphite lye emulsion, at 0·05 per cent. The results are given in Tables V and VI.

TABLE V.

DDT RESIDUES ON BLACKCURRANTS, 1952 :
DDT APPLIED AT AN EARLY STAGE IN FRUIT DEVELOPMENT.

Date of sampling	Condition of fruits	Average weight of fruit (g.)	DDT p.p.m.	DDT mg./100
19 May	Small, green ..	0·23	29	0·66
27 May	Green	0·25	20	0·52
18 June	Large, green, some black ..	0·48	7	0·32
3 July	Large, black ..	0·78	3	0·22

TABLE VI.

DDT RESIDUES ON BLACKCURRANTS, 1952 :
DDT APPLIED AT A LATE STAGE IN FRUIT DEVELOPMENT.

Date of sampling	Condition of fruits	Average weight of fruit (g.)	DDT p.p.m.	DDT mg./100
23 June	Large, green, some black ..	0·56	16	0·88
3 July	Large, black ..	0·73	8	0·54
21 July	Large, black ..	0·85	6	0·51

318 hours of sunshine and 4·2 ins. rain were recorded during the period 19 May to 3 July, and 198 hours sunshine and 0·66 ins. rain from 23 June to 21 July.

Tomatoes. Trials were made under glass using the benzene-sulphite lye emulsion at 0·05 per cent DDT. Application was made when the fruits were very small, of average weight 3 g. They then showed 6·8 p.p.m. DDT. Seven weeks later when the fruits were red and averaging 66 g. in weight, the deposit was 0·3 p.p.m. Under glasshouse conditions, with no possibility of loss from rainfall, the fall in the residue, as p.p.m., was thus almost exactly proportional to the increase in weight of the fruits.

The detection of DDT deposits.

A modification of the method with which this work was done may be used to detect DDT deposits on plant surfaces. The initial extraction with carbon tetrachloride, the passage through alumina, the removal of the solvent and the nitration of the residue are carried out as before. After nitration, the solution is diluted with water when a turbidity indicates the presence of DDT.

The solution is made alkaline to litmus with 9N sodium hydroxide solution, extracted with ether, the ether is removed to low volume and 1 ml.N alcoholic potash is added. Confirmation of the presence of DDT is shown by the formation of a blue colour.

A distinct turbidity and blue colour are given by 0·05 mg. DDT. equivalent, on a 5 g. sample of leaves, to 10 p.p.m.

Further work is in progress to use the test on a quantitative basis and to combine with it a method for BHC residues, so permitting the examination of deposits from mixed DDT-BHC sprays.

COPPER

Outdoor tomatoes may be sprayed with copper preparations a number of times throughout the growing season to control blight. Copper is also used, with petroleum oil, against *Cladosporium* on tomatoes under glass. In view of the recently suggested tolerance of 20 p.p.m. Cu for fruits and fruit products such as chutney, tests were made to determine to what extent copper residues were likely to build up following successive applications of the fungicide.

Method of analysis. The sodium diethyldithiocarbamate method was used. The plant material was ashed, the ash was taken up in nitric acid, the solution diluted and made alkaline with ammonia, heated, filtered and made up to volume. Aliquots were treated with the dithiocarbamate reagent and the colour produced was compared with standards prepared from copper sulphate.

Tomatoes under glass. Plants were treated with a proprietary copper oxychloride, with added wetter, at two levels of Cu, 0·125 and 0·25 per cent. Fruits averaging 53 g. in weight were present. A fortnight later, when the fruits averaged 67 g., second applications were given, again at 0·125 and 0·25 per cent Cu. The plants were sprayed to run-off, with wetting of both surfaces of the leaves. Samples of leaves and fruits were taken for analysis as soon as the deposits had dried. The results, showing the cumulative effect in the absence of weathering of two applications at the 0·125 and 0·25 per cent levels of copper, are given in Table VII.

TABLE VII.

COPPER RESIDUES ON TOMATOES UNDER GLASS, 1951.

Date of application	Treatment. % Cu	Cu p.p.m.					
		Leaves			Fruits		
19 Sept. ..	Wetter alone ..	3,	3,	3	<0·1,	<0·1,	<0·1
	0·125 once ..	380,	256,	370	1·5,	3·5,	3·0
	0·25 once ..	574,	737,	767	5·5,	4·7,	5·0
2 Oct. ..	0·125 twice ..	595,	756,	698	4·9,	7·0,	3·8
	0·25 twice ..	2430,	1880,	1984	21,	23·5,	22·5

Outdoor tomatoes. In 1952 a trial was made to study the build-up and persistence of copper deposits on outdoor tomatoes subjected to normal weathering. A proprietary colloidal copper preparation, with added wetter, was used at the rate recommended commercially (0·06 per cent Cu). Different sets of plants received one, two, three or four treatments, made at approximately fourteen-day intervals. Deposits were determined immediately after each application and the persistence of the deposits during the intervals between treatments was also recorded. The fluctuations in deposits on foliage and fruits during the experiment are given in Table VIII.

TABLE VIII.

COPPER RESIDUES ON OUTDOOR TOMATOES, 1952.

Date of application	Treatment	Date of sampling	Average weight of fruit (g.)	Cu p.p.m.	
				Leaves	Fruits
29 July ..	Wetter alone	29 July ..	31	5, 6	0·1, 0·1
		15 Sept. ..	57	2, 4	0·1, 0·1
29 July ..	Colloidal copper	29 July ..	31	56, 103	0·7, 0·8
		13 Aug. ..	35	— —	0·2, 0·2
		1 Sept. ..	46	17, 15	0·1, 0·2
		15 Sept. ..	57	6, 13	0·1, 0·1
13 Aug. ..	Colloidal copper	13 Aug. ..	35	— —	1·5, 1·8
		1 Sept. ..	46	52, 45	0·4, 0·4
		15 Sept. ..	57	14, 15	0·1, 0·1
1 Sept. ..	Colloidal copper	1 Sept. ..	46	151, 171	3·2, 2·5
		15 Sept. ..	57	71, 86	0·1, 0·1
15 Sept. ..	Colloidal copper	15 Sept. ..	57	113, 86	1·4, 0·4

The rainfall recorded for successive intervals was as follows : 29 June to 12 Aug., 4·4 ins. ; 13 Aug. to 31 Aug., 1·8 ins. ; 1 Sept. to 14 Sept., 2·0 ins.

Discussion.

The method used for the determination of DDT deposits is sensitive and needs no special apparatus or reagents. The initial extraction of the residues was carried out by successive soaking and agitation with portions of hot solvent and was sufficiently drastic to remove surface and underlying deposits. The values for the residues recorded in the tables are given to the nearest p.p.m. Replicated samples showed surprisingly little variation in the deposits found, particularly at the low levels of contamination. There is however the possibility that the DDT may have penetrated within the fruits and this has not yet been examined.

Armstrong and his colleagues (1952) have developed an analytical procedure very similar to our own for the examination of the penetration of insect cuticle by DDT and related compounds. We have also successfully applied our method to the estimation of DDT residues in grain.

Information has been gained on the levels of initial residues on soft fruits likely to result from application at the rates used commercially and on the extent to which the fall in the levels of residues may be associated with growth and weathering. The initial residues on small soft fruits after spraying with 0·1 per cent DDT will vary with the thoroughness of application, but from one treatment may be expected to be of the order of 20–40 p.p.m. There has been no indication from this and other work that the type of formulation (i.e. dispersible powder or emulsion) influences to any major extent the initial deposit retained. As the fruits develop, the deposits, as p.p.m., decline at a greater rate than may be accounted for by the growth factor alone. Weathering clearly plays a part, as seen from the values for actual DDT retained by the fruits.

Climatic factors that are likely to contribute to the weathering of DDT surface spray deposits are rainfall, leading to mechanical loss, and light and temperature inducing oxidation and, perhaps to some extent, volatilization of the DDT. In 1952, the decline in residues on gooseberries was greater than that in 1951, possibly associated with the higher rainfall occurring during the second year. In contrast, little actual DDT was lost from tomatoes under glass. While the evidence is insufficient to permit an assessment of the relative effect of the different factors in reducing deposits, loss by rainfall, under our conditions, may well be important.

An indication was obtained, from this and other work on shoots from apple rootstocks subjected to normal weathering and controlled leaching, that DDT tends to be lost from deposits of dispersible powders rather more easily than from deposits of emulsions. The effects obtained may depend upon the particular products used, and no generalization can be made on this point. There is, however, no doubt that DDT is a persistent insecticide whether applied in dispersible powder or emulsion form, approximately one-third of the initial deposits still being retained after five weeks of appreciable sun and rain intensity.

Where long periods occurred between the application of DDT and the harvesting of the fruit, the combined effects of growth and weathering resulted in final DDT residues well below 7 p.p.m. Allen and his co-workers (1950) in Canada have recorded final DDT deposits on gooseberries, after one application, of about 3 p.p.m., values very similar to our own. Higher levels of contamination may occur if soft fruits are sprayed at a late stage in their development, but the risk does not appear to be excessive. The increase in the weight of the fruit with growth is progressively greater than the increase in its surface area ; the initial residues as p.p.m. on large fruits will therefore be lower than those on fruits sprayed when small. Offsetting this advantage is the shorter period between spraying and harvesting during which weathering and the growth factor exert their influence. These effects are seen in the experiments on blackcurrants sprayed with DDT at 0·05 per cent at two stages of fruit development. The small fruits held about 30 p.p.m. DDT falling eventually to 3 p.p.m., while fruits sprayed when three-quarters grown showed 16 p.p.m. DDT initially and 6 p.p.m. when harvested. The contamination of apples by DDT has interested us in view of the fact that it may be used against codling moth. High values as p.p.m. are likely to be precluded because of the large mass of the fruit in relation to surface area ; the actual deposit on each fruit, however, may be significant and this fact should not be overlooked.

In work on copper sprays for loganberries, Shaw (1936) has shown that no apprehension need arise, even when application is made shortly before picking. Our preliminary trials on tomatoes have indicated that appreciable residues may build up when sprays containing 0·125 or 0·25 per cent are used in the absence of weathering. On outdoor plants under wet conditions much of the deposit of the particular form of copper used was lost between successive treatments.

One feature of the results, with both DDT and copper, has been the high deposits, as p.p.m., held by leaves compared with fruits on subjecting both to the same spray. This is merely an expression of the much greater surface area, with leaves, per unit of weight. The matter may, however, become important should foliage crops be treated with a persistent and potentially dangerous material.

Much work remains to be done, for example, on the effect of repeated treatment, on the influence of spray formulation and of the nature of the surface treated on the extent and persistence of residues, and in correlating chemical data with biological tests. The general pattern of residues however is beginning to unfold and the position so far is reassuring.

Summary.

1. The levels and persistence of DDT residues on gooseberry, raspberry, loganberry, blackberry, blackcurrant and tomato and of copper residues on tomato have been examined following application at the rates used commercially.
2. Little risk occurs of significant DDT residues provided that application is made at an early stage of fruit development.

Acknowledgment.

We are indebted to Mr. J. H. Russell and Mr. C. W. Harper who applied the materials.

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SPRAY APPLICATION PROBLEMS.

III. A SEDIMENTATION METHOD FOR THE DETERMINATION OF THE EFFECTIVE PARTICLE SIZE DISTRIBUTION OF DISPERSIBLE POWDERS.

By W. D. E. THOMAS and A. J. SIMS.

Introduction.

Dispersible powders consisting of 15–50% DDT, BHC, or other active principle, an inert carrier, and a wetter provide a convenient method of using insecticidal preparations. In the field the procedure is to cream the powder in a bucket and to add it to the bulk of water. Since a homogeneous suspension is desirable during spraying it is important that the suspension should possess reasonable stability. This is ensured in many spraying machines by constant agitation, but on a small scale (e.g. knapsack sprayer), it is dependent on the active ingredient remaining uniformly dispersed. Should the suspension settle out quickly, the composition of the issuing spray would vary over wide limits with unequal deposition on the foliage. Consequently insect control would become extremely variable.

A sedimentation performance test is thus required to assess the physical stability of aqueous suspensions of dispersible powders. Such methods have been suggested in the past, but, in general, they have involved only simple sedimentation in a graduated cylinder with visual examination of the sediment and with little consideration for the content of active principle. A more precise method has been developed by Orrell (1949) for BHC, and another method for DDT has been suggested for acceptance by the Expert Committee on Insecticides of the World Health Organisation of the United Nations (Anon. 1951).

The modified Andreasen method described below has been developed particularly for DDT dispersible powders. It can however be adapted for the examination of other dispersible powders provided a convenient method is available for determining the active principle present. The method requires standard laboratory equipment only, and is capable of routine use. Comparative analyses have been carried out with it in several independent laboratories and good agreement has been obtained between the different workers testing the same products.

Principle of the method.

A 1% dispersion of the powder is prepared in a $2\frac{1}{2}$ l. winchester, and allowed to settle under quiescent conditions. 50 ml. samples are withdrawn from a fixed level in the suspension after 1, 3, 20 and 75 min. respectively from the beginning of settling. The DDT content of each sample withdrawn is determined, and its corresponding limiting Stokes' diameter calculated. The effective size-distribution analysis of the DDT can then be expressed in the form of a Stokes' diameter (ds) – weight % under size ds relation. When these data are plotted on a graph the particle size distribution of the DDT can be seen at a glance.

Method.

25 g. of the DDT dispersible powder is creamed in a beaker using a glass rod, with an aliquot (about 50 ml.) from $2\frac{1}{2}$ litres of "hard" water prepared as described in the M. of A. Tech. Bull. No. 1. 2 l. of the "hard" water is poured into a $2\frac{1}{2}$ l. winchester and the cream added, the remainder of the $2\frac{1}{2}$ l. water being used to ensure completeness of transfer. The winchester should be reasonably vertical and preferably be non-fluted inside. A strip of paper about $\frac{1}{2}$ in. wide and 9 in. long is stuck along the height of the bottle to permit the surface level of the suspension to be marked after each sampling.

The suspension is thoroughly dispersed by inverting the winchester 20 times through 180° avoiding frothing as much as possible ; the winchester is then placed on a level bench and a stop watch started simultaneously. The surface level of the suspension is marked on the paper (h_0) and after 50 sec. a 50 ml. pipette (Fig. 1) is quickly lowered into the winchester until it rests on the neck. The free end is joined to a 2-way tap A and this in turn by rubber tubing to a 3-way tap B for releasing pressure. Suction is provided by a water pump and is controlled by the depth of immersion of the glass tube (drawn out tip) into mercury. The precise depth of immersion (approximately 8 cm.) is varied until the time of filling the pipette with any 1% DDT dispersible suspension is 20 ± 1 secs. Once set for one such operation no further alteration is necessary to maintain a time of 20 ± 2 secs. for filling. Temperature need not be controlled to any precise value within the range 15–25° C. but it should be maintained as constant as possible during a test.

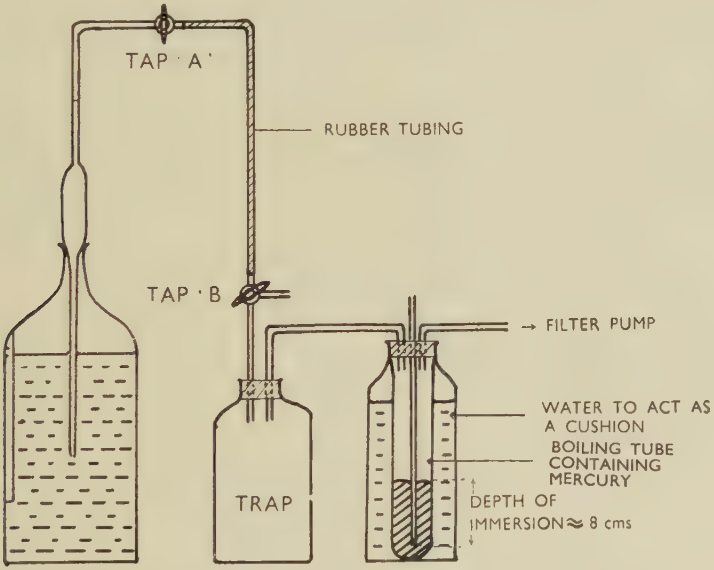
TAP 'A'
RUBBER TUBING
TAP 'B
→ FILTER PUMP
WATER TO ACT AS A CUSHION
BOILING TUBE CONTAINING MERCURY
DEPTH OF IMMERSION ≈ 8 cms
TRAP

Fig. 1.

The stem of the pipette should be such that the tip comes 12–16 cm. below the surface. Over-filling the pipette is easily avoided by closing tap A when the level of the rising suspension is about $\frac{1}{2}$ in. below the mark. The sample is transferred to the first of four 100 ml. beakers and the new level of the suspension marked on the paper (h_1).

Three subsequent samples at 3, 20 and 75 min. respectively are taken : pipette filling is begun 10 sec. before each of these times. After the 4th sample has been taken and the level (h_4) marked, suction is continued through the pipette to waste until the level has dropped to that of the pipette tip (h_5). The suspension can alternatively be syphoned to waste (through a rubber tube) until the surface level is within a cm. of the pipette tip. Withdrawal of the suspension is then continued into the pipette until the surface breaks at the level of the tip.

For each sampling the average height of surface level above the sampling point is measured, e.g. for the first it is $\frac{1}{2}(h_0 + h_1) - h_5$ cm.

Four sintered glass crucibles (No. 4 grade) are successively extracted with hot water and benzene, dried, cooled and weighed. The samples are filtered (filtration is considerably facilitated by allowing the suspension to settle overnight) and the supernatant liquor decanted through the crucible before the precipitate is transferred. The crucibles are dried for 2 hr. at 85° C., cooled, weighed and the DDT extracted from each with 50–75 ml. warm benzene. The crucibles are redried at 85° C. for 1 hr., cooled and weighed. The DDT content of each sample is given by the observed loss in weight of the crucibles. The whole of the sedimentation test should be carried out in duplicate and close agreement (within 5%) obtained between the data.

The original composition of the DDT wettable powder is determined in a preliminary experiment by successively extracting a weighed amount (0·5 — 1·0 g.) with warm water and benzene through a No. 4 sintered glass crucible, and weighing after each stage. The loss in weight with water gives the wetter content, and with benzene the DDT content. The carrier concentration is given by difference.

Calculation.

1. *Composition of DDT wettable powder.*

Wt. of DDT wettable taken $= a$ gm.
Wt. of crucible $= x$ gm.
Wt. of crucible + powder after water extraction $= y$ gm.
Wt. of crucible + powder after benzene extraction $= z$ gm.

$$\therefore \text{ \% wetter} = \frac{a-(y-x)}{a} \cdot 100\,; \qquad \text{\% DDT} = \frac{y-z}{a} \cdot 100$$

$$\text{\% carrier} = \frac{z-x}{a} \cdot 100$$

2. *Particle size distribution.*

For a system of particles of density σ sedimenting in a quiescent fluid medium of density ρ the maximum size of particles (diameter ds) present at a depth h below the surface after any time t from the commencement of settling is given (assuming the applicability of Stokes' Law) by

$$ds = 175 \sqrt{\frac{\eta}{\sigma - \rho} \cdot \frac{h}{t}}$$

where

d_s = Stokes' diameter of particles (in microns).
η = viscosity of fluid (in poises).
σ = S.G. of sedimenting powder.
ρ = S.G. of fluid medium.
h = mean height of surface above sampling point (cm.).
t = time of sampling after commencement of settling (mins.).

A sample withdrawn from a depth h after a time t from the commencement of settling will contain all the particles in the original powder up to a size d_s, i.e. the sample will be identical with the original material but with all particles greater than d_s removed.

Applying this general equation to the DDT constituent of a DDT wettable powder sedimenting in a dilute aqueous suspension ($\sigma = 1{\cdot}54$, $\rho = 1{\cdot}0$ and $\eta = 0{\cdot}01$ for water at room temperature (20° ± 5°) the general equation reduces to :—

$$d_s = 23{\cdot}8 \sqrt{\frac{h}{t}}$$

and the % of particles under size d_s at each of the four sampling times is given by

$$d_s = \frac{\text{Wt. of DDT in the 50 ml. sample}}{\text{initial conc. of DDT per 50 ml. suspension}} \times 100$$

Data are tabulated as follows : e.g. for a DDT wettable powder which contains (by analysis) 49·5% DDT

Wt. of DDT wettable taken = 25·00 gm.
DDT content of suspension for uniform dispersion = 0·247 gm./50 ml.

No. of sampling	Time of sampling (mins.)	Depth of sampling (cm.)	h t cm./mins.	$d_s = 23{\cdot}8 \sqrt{\frac{h}{t}}$ (micron.)	Wt. of DDT in sample (gm.)	% DDT under size d_s in sample
1	1	15·4	15·4	96·4	0·230	93·5
2	3	14·95	4·98	53·1	0·200	81
3	20	14·4	0·72	20·2	0·080	32·4
4	75	13·9	0·185	10·2	0·030	12·1

Typical curves for weight per cent under size at each level of Stokes' diameter for the DDT and inert carrier components of two commercial dispersible powders are shown in Fig. 2. Comparing the DDT components of the two powders it is seen that whilst 95% by weight of the DDT in one powder is under an effective size of 80 μ (curve C), only 37% by weight of the DDT in the other powder is under 80 μ (curve A).

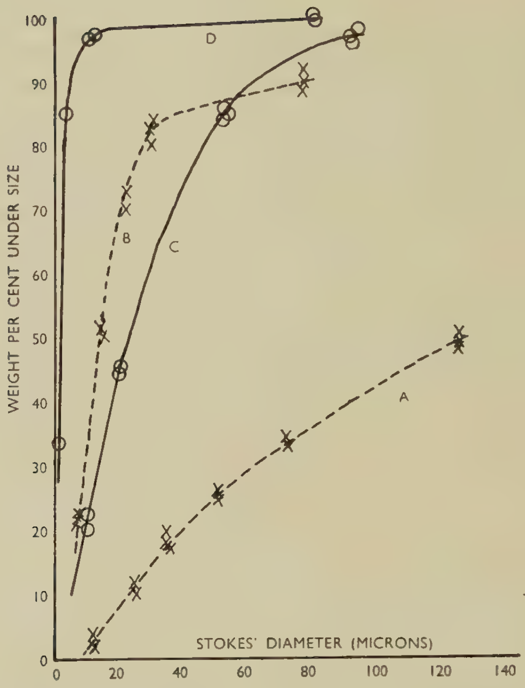

Fig. 2. Particle size distribution (weight per cent under size) curves for the DDT and Carrier components of two commercial dispersible powders.

Curves A and B show size distribution of DDT and Carrier respectively in a powder of poor suspending properties.

Curves C and D show size distribution of DDT and Carrier respectively in a powder of good suspending properties.

Discussion.

It is desirable in all sedimentation methods for determining particle sizes that the particles should settle without mutual interference and also that they should obey Stokes' Law. Unless the suspension is sufficiently dilute the particles will interfere with each other and some of the finer particles might well be carried down by the more rapidly falling coarser ones. A concentration of 1% provides a reasonable compromise between what is desirable and what is practicable in order to get weighable deposits in the samples. Free settling may also be disturbed by the setting up of convection currents through temperature gradients within the suspension. This effect can be minimised by maintaining the temperature as near as possible to its value at the beginning of the test.

Stokes' Law is strictly applicable only when the flow is entirely viscous. The nature of the flow can be expressed quantitatively in terms of a dimensionless quantity, the Reynolds number (Re), the ratio of inertia forces to viscous forces in the system. For small values of Re (up to 0·2) the resistance to flow is purely viscous and Stokes' Law can be safely applied. For the first sampling the Re value is usually higher than 0·2 but this determination is in any case subject to greater errors than the later ones because of the short period of quiescent settling which precedes sampling. This sampling after 1 min. is nevertheless retained in the method because it gives an indication of the proportion of coarser particles in the suspension.

The Stokes' diameter d_s value for a particle, or group of particles, calculated from sedimentation observations, may be defined as the diameter of a sphere of the same density having the same settling velocity as the particles. It approximates closely to the true particle diameter only if the sedimenting particles are nearly spherical in shape. For DDT wettable powder-water systems, this theory must be applied with considerable care because it is by no means established that the DDT particles fall freely—the settling particles may well be heterogeneous, consisting of DDT and carrier, the DDT possibly as a surface coating on the carrier particles. Even if the DDT particles are homogeneous, they may still be agglomerates. The initial dispersion, simulating conditions in the field, may be insufficient to produce complete dispersion of the powder. When therefore it is found by the sedimentation test that the % DDT particles below 50 μ diameter in a wettable powder is, say, 20%, what is implied is that 20% of all the DDT in the system behaves in the same way as 50 μ spherical DDT particles allowed to fall freely. The actual DDT or DDT-carrier particles may be quite different from this.

The sedimentation test however provides information concerning the way DDT wettable powders behave under normal field conditions. No consideration has been given in this work to the optimum particle size required for insect control. The method is such that with a suitable modification for determining the active principle it should be applicable to the analysis of other dispersible powders.

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SPRAY APPLICATION PROBLEMS: IV. PRELIMINARY FIELD EXPERIMENTS ON THE CONTROL OF APPLE SCAB BY SMALL-VOLUME SPRAYS.

By H. G. H. KEARNS, R. W. MARSH and N. G. MORGAN.

The application of fruit sprays as droplets impelled by a high-velocity air-stream was first developed for use with insecticides. The use of this method for fungicides is more recent, but in U.S.A. progress reports have been published on the results of low-volume fungicide applications for the control of apple scab. Burrell (1951) has stated that preparations of dispersible sulphur have successfully controlled apple scab in New York State when used at up to eight times the standard concentration and one-eighth volume. He advises growers, however, not to exceed the 4x concentration.

In England, M. H. Moore (1948) has shown that careful application of undiluted lime sulphur to dwarf pyramid apple trees results in only slight damage. The rate of application of the undiluted material suggested by Moore for plantations of bush apples is ten gallons per acre.

In the experiments made at Long Ashton in 1952 the two principal aims were: (a) to compare the performance of a conventional large-volume 1% lime sulphur spray with that of 2% lime sulphur at half volume and of 4% lime sulphur at quarter volume; (b) to compare the performance of the large-volume lime sulphur spray with that of a small-volume application, using a concentration of 1% for *both* treatments. Comparison (a) was made on Worcester Pearmain apples and comparison (b) on Cox's Orange Pippin.

Plots.

The Worcester Pearmain trees used were in Plot 23 of the Long Ashton plantations and formed a block of 240, planted in 1935 at 15 ft. on the square, with Laxton's Superb pollinators (see Fig. 1). These trees had been shaped to suit automatic spraying.

The Cox's Orange Pippins, planted in 1926, comprised nineteen trees in three rows in Plot 14 of the Long Ashton plantations. This plot is being used for a tree shaping trial fully described in the 1950 Long Ashton Annual Report (Jarrett 1951). The trees, originally at 15 ft. on the square, have now been thinned to a diagonal plant of approximately 21 ft. (see Fig. 2). The remaining varieties on this plot, Worcester Pearmain, Edward VII and Crawley Beauty, were included in the spraying trial, but records were taken only on Cox's. These trees were not well suited for automatic spraying.

Spray materials.

Throughout the trials lime sulphur at the concentrations stated in the paragraph on lay-out of experiment was used.

At petal-fall on plot 14, 0·006% gamma BHC was added to the lime sulphur for the control of apple sawfly. The gamma-BHC was dissolved in benzol and emulsified with sulphite lye: 0·1% of Limpsopon 40 wetter was added.

Machines and Method of Application.

A conventional type of tractor-drawn power-take-off-driven spraying machine, supplying a Long Ashton standard 3-nozzle short-range broom with 5/64″ disc apertures at 400 lb. per sq. in. pressure, was used for the large-volume hand applications. Particular attention was paid to thorough spraying and extensive run-off of excess wash occurred.

The small-volume applications were made with an "air-flow" machine identical with that described (Kearns and Morgan, 1950) in respect of the axial flow fan, power unit, pump, nozzles and general lay-out. The main modification made was the insertion, in the fan delivery trunking, of a 19″ diameter cone with its apex pointing inwards and its base projecting outside. This directed the air to the peripheral area of the trunking and produced a wider air swathe with little loss of air volume or speed. Spray liquid from the nozzle rings impinged on the cone surface and was then sheared off into droplets by the passing air stream.

The delivery trunking was adjusted so that the air stream was directed rearwards at an angle of from 45° to 50° from the direction of travel, depending upon wind conditions. On plot 23, where the Worcester trees were of limited height and depth, the air swathe was just wide enough to give complete cover, while on plot 14 continual vertical movement of the trunking by a hand capstan was necessary to cover trees of varying height and density.

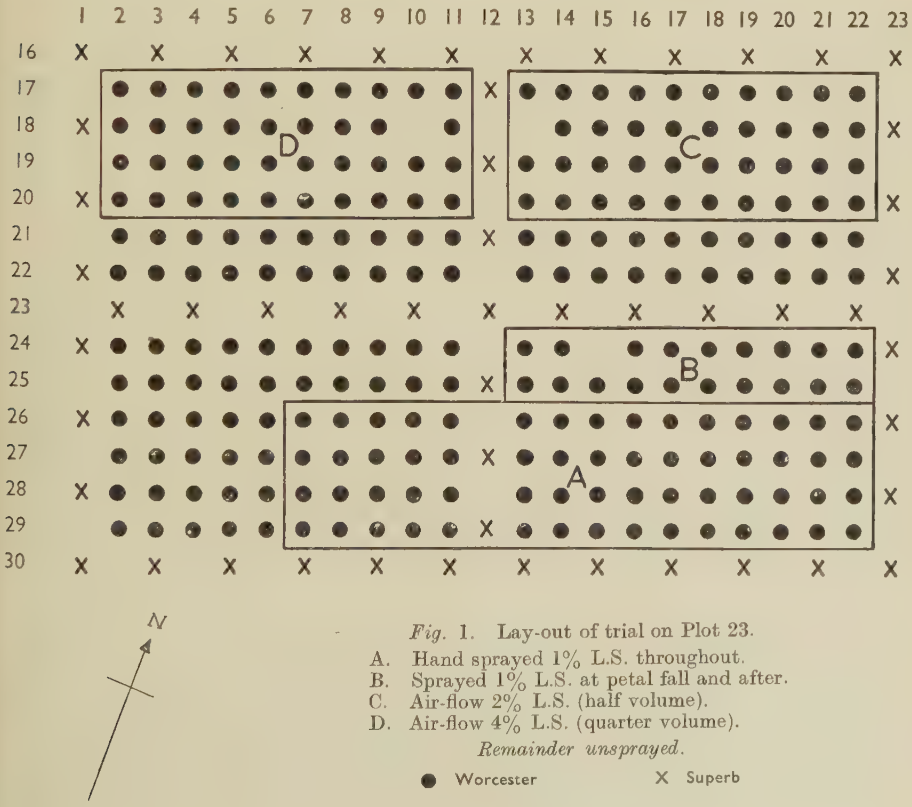


Fig. 1. Lay-out of trial on Plot 23.

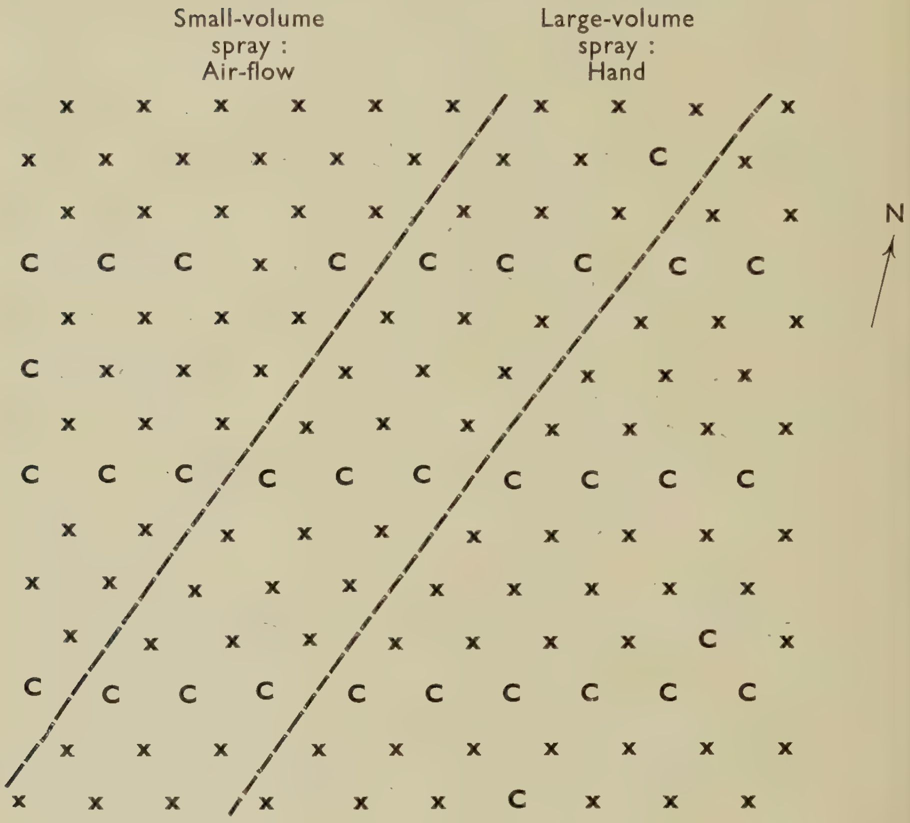
A. Hand sprayed 1% L.S. throughout.
B. Sprayed 1% L.S. at petal fall and after.
C. Air-flow 2% L.S. (half volume).
D. Air-flow 4% L.S. (quarter volume).
Remainder unsprayed.

● Worcester X Superb

Fig. 2.

LAY-OUT OF TRIAL ON PLOT 14.

C = Cox, x = other varieties.



The rate of application was adjusted by means of nozzle disc apertures and tractor speeds. The " air-flow " application of normal dilutions of lime sulphur on plot 14 was fixed at approximately 100 gallons per acre, i.e. approximately 1/6th of the volume applied by hand. It should be emphasised, however, that the volume of spray materials applied per acre by any automatic spraying method has little significance without consideration of tree shape, size and density since, in a conventional fruit plantation with considerable spaces between the trees, one-quarter to one-third of the total volume emitted from the machine may not reach the foliage directly but by drift.

Lay-out of experiments and spraying dates.

In experiments with small-volume spraying, unless a very large area of uniform trees is available, replication of treatments is not feasible, because of the problem of spray drift. In the Long Ashton trials of 1952, therefore, the blocks for low-volume treatment were chosen on the leeward side of the plots and the large volume treatments were given on the south (windward) side.

Worcesters (see Fig. 1). Two blocks of 40 trees each (C and D) on the north side of the plot were sprayed respectively with 2% lime sulphur at half the volume applied by lance and 4% lime sulphur at one quarter of the volume applied by lance on the conventionally sprayed plot. These treatments were applied with the air-flow machine on 13th May, 20th May and 4th June. Sixty trees at the south of the plot (A in Fig. 1) received 1% lime sulphur on the same dates, at the rate of 400 gallons per acre. The lime sulphur applied to Section A on 4th June was included in a combined wash of 1% lime sulphur, 0·05% nicotine and 0·1% Limpsopon 40 wetter. The nineteen trees adjoining (Section B in Fig. 1) were sprayed only with 1% lime sulphur at petal fall and after. Sections A and B were sprayed by a conventional machine using hand-directed lances with 5/64″ aperture discs. The remaining eighty Worcester trees in the plot had no fungicidal spray.

Cox's (see Fig. 2). The ten Cox trees included in a diagonal NE-to-SW strip on Plot 14 were very thoroughly sprayed with 1% lime sulphur (plus wetter), at the rate of 650 gallons per acre on 5th May, 21st May, 7th June, 19th June and 7th July. On the same dates the nine Cox trees west of the strip received 1% lime sulphur at 100 gallons per acre, applied by the air-flow machine. No trees were left unsprayed on this plot.

Weather.

Although the summer of 1952 was dry in many of the fruit-growing districts in England, the rainfall at Long Ashton in the period May–September was $4\frac{1}{4}$ in. above the mean. Except for July, each of these months was wetter than average, the totals being (in inches): May 4·28, June 3·94, July 0·77, August 6·16, September 3.23. The season was, therefore, favourable for apple scab infection at Long Ashton and there were heavy attacks on the unsprayed trees.

Methods of recording.

On Plot 14, records were taken of every tree of Cox included in the trial. Mean percentage scabbed area per leaf was computed from samples of 250 leaves per tree taken on 24th July. At the same date, fruit numbers per tree were taken.

On Plot 23, sixteen trees were recorded, being chosen at random as samples for each of the treatments (unsprayed, 1% l.s., 2% l.s. half volume and 4% l.s. quarter volume). The mean percentage scabbed areas for leaf samples taken on 23rd July were computed as above. On 5th August fruits per tree were counted, and the percentage scabbed areas for the fruit computed for samples of 100 apples per tree. (On trees that had received 4% lime sulphur it was found necessary to take fruits from a group of trees to make up a total of 100).

Results.

The summarised results of the counts are assembled in Tables I and II.

Both tables show that the hand-directed large-volume spray gives the highest level of apple scab control. However, the control obtained by the small-volume applications would be considered satisfactory in commercial practice. Table I shows a very heavy scab attack on the unsprayed trees, which is reduced to one-sixth by the 2% half-volume spray. A somewhat

TABLE I.

RESULTS ON WORCESTERS

(means from four trees per treatment).

Treatment	Leaves (23rd July)	Fruit (5th Aug.)		
	Mean % scabbed area	Mean % scabbed area	No. per tree	% russeted
A. Unsprayed	2·80	3·15	>100	39
B. Hydraulic 1% l.s. (400 g.p.a.) ..	0·21	0·09	>100	74
C. Air-flow 2% l.s. (½ vol. of B.) ..	0·53	0·51	100	14
D. Air-flow 4% l.s. (¼ vol. of B.) ..	0·36	0·22	20	27

Significant differences in scab control : B, C, D $>$ A ; B $>$ D.
„ „ „ yields : A, B, D $>$ C.

TABLE II.

RESULTS ON COX

(means of ten trees per treatment : 24th July).

Treatment	Leaves. Mean % scabbed area	Fruit. No. per tree
A. Hydraulic 1% l.s. 650 g.p.a. ..	0·03	171
B. Air-flow 1% l.s. 100 g.p.a. ..	0·29	347

better scab control is given by the 4% spray but this strength has clearly caused serious fruit drop, the yield per tree on block C being less than one-fifth that of any block. While this result is obtained from an unrandomised experiment, it is not considered that so striking a difference could be due to any factor other than the spraying treatment.

In Table II it might appear at first sight that this result is reversed, as the trees given the high-volume spray have yielded only half the crop of those receiving the air-blast application. However, on these trees the small-volume spray was not at increased concentration, so it is possible that the total sulphur retention was less than that on the Cox's given the very thorough large-volume application. The latter show almost perfect scab control on the foliage, significantly better than that resulting from the small-volume spray. However, the scab control in this " small-volume " block, both on leaves and fruit was commercially satisfactory.

As this experiment included no control trees, the merits of the small-volume spray treatment on Plot 14 cannot be precisely assessed, but the absence of phytocidal effect on the sensitive variety, Cox, is noteworthy. If further trials confirm the practicability of using small-volume lime sulphur sprays without increase of concentration by means of " air-flow " methods a new field will be opened in treatments against apple scab.

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SPRAY APPLICATION PROBLEMS:
V. THE AUTOMATIC LOW-PRESSURE SPRAYING OF SMALL BUSH FRUIT.

By H. G. H. KEARNS and N. G. MORGAN.

Lance spraying of fruit crops is expensive, largely due to the slowness of the operation and the consequent high cost of labour. For this reason, increasing attention is being given to methods of applying the sprays with mobile automatic machinery.

Unfortunately automatic spraying of large-volume (run-off type) washes can only be efficiently done if the crop is of suitable size and shape. For hydraulic spraying of large bush apple trees, the flow pressure must be not less than 250 lbs. per sq. in. This is because of the necessity of propelling the droplets to the top and through at least three-quarters of the tree. Blackcurrants and dwarf-pyramid bush trees are much more readily sprayed, as their size and shape do not demand high pressure and all the foliage is near the spray nozzles. The effective cover obtained from droplets applied at 75 lbs. p.s.i. and travelling 3–4 feet is equal to that obtained from droplets applied at 400 lbs. p.s.i. travelling 15 feet. Blackcurrants are unpleasant crops to spray with lances as there is much manual work associated with pulling long hoses along rows over arable land and this results in high labour costs and undue waste of wash.

Dwarf-pyramid apples, and blackcurrants, are well suited to spraying with hydraulic automatic machinery employing low flow pressures. An outfit for this work must be fitted with a large-capacity pump, but has to be narrow enough to pass between rows planted 8 ft. apart. The large size, weight and expense associated with a reciprocating pump can be avoided by using a gear or other type of small pump. A reciprocating pump would cost £200 compared with a gear pump at £25 of similar throughputs. Experience suggests that the gear pump will maintain its efficiency for about 400 hours of use when applying oil emulsions, lime sulphur and finely divided copper compounds. The original efficiency can be regained with an overhaul costing £10.

A small automatic sprayer hauled and driven by a narrow-track wheeled tractor and employing a gear pump was used successfully for spraying blackcurrants and pyramid apples.

The automatic spray machine (Plate I). The spray machine consists of a chassis on two pneumatic-tyred wheels, gear pump and countershaft, very large suction filter, water raiser, agitator, spray gates and tractor power-take-off drive.

The chassis consists of a light-channel, electrically-welded structure fitted with an axle with one pair of 16″ × 5″ or 19″ × 6″ pneumatic-tyred wheels, the axle located so that the machine is heavy forward on the draw bar of the tractor. The overall width of the axle and wheels is 46 ″. Detachable wheel guards are provided, to prevent damage, in particular to blackcurrant shoots. On the forward portion of the chassis a countershaft and pump is fitted.

The tank. A tank of not less than 110–120 gallons capacity is necessary, a convenient size being 48″ long × 28″ wide × 30″ deep. It should be fitted with an anti-splash filler hole of not less than 18″ diameter, and a drop-in type filter basket at least 6″ deep fitted with a Monel-metal or other non-corrosive gauze of 24-mesh. The gauze should be fitted as a replaceable collar over a row of 3″ diameter holes in the side walls of the basket. All mild steel parts should be hot-dipped galvanised after manufacture. A larger tank of 210 gallons capacity reduces considerably the time expended in filling up, but the tank size can only be increased vertically and the top of a 210-gallon tank is about 6 ft. from ground level. This causes difficulty in filling, so a standing bracket and a holding rail must be fitted at convenient heights at the rear of the tank.

Gear pump. The spray machine will be required to apply up to 500 gallons per acre, on apple pyramids 8 ft. high, at a tractor speed of about 2 miles per hour. To meet these requirements the pump delivery must be about 20 gallons per minute at 75 lbs. per sq. in.

The gear pump should be made of phosphor bronze throughout with stainless-steel shafts and preferably with self-lubricating bearings. A well-designed pump fitted with gears of 3″ long × 2½″ diameter and running at 900–1,000 r.p.m. is satisfactory. The suction piping should be of 1″ bore and fitted with a very large efficient filter, e.g. a tubular filter 24″ long × 4″ bore with a tubular gauze element of 32 B.S.S. mesh gauze. The delivery piping must be fitted with a simple adjustable relief valve discharging back into the bottom of the tank, and the valve should be fitted with a phosphor-bronze seat and ball. Two take-off hose points are fitted to the rear panel of the tank, each point being controlled by a quick-acting tap accessible from the seat of the tractor, so that one or both spray gates can be operated. A water raiser, used for filling the tank at the rate of 20 gallons per minute, is fitted into the high-pressure circuit and controlled by a valve. It carries a 20 ft. length of 1½″ bore suction hose and discharges into the tank filter basket. The pump is driven from a ball-bearing countershaft assembly by means of an adjustable duplex 0·375 roller-chain assembly running in a chain cover. The gear ratio to the pump is adjusted to provide 900–1,000 r.p.m. The countershaft is fitted with the universal joint of the tractor telescopic power-take-off shaft which generally runs at 400–450 r.p.m. under load. The tank has an agitator consisting of a shaft with a single paddle fitted into substantial lubricated stuffing boxes : the speed should be about 100 r.p.m. and it is driven from the countershaft of the pump drive.

The spray nozzle gates (Plate II). Two adjustable gates are fitted to a channel-iron pillar attached to the middle of the rear panel of the tank. The gates on the outside vertical edge have adjustable clamp brackets, holding a ¾″ bore copper tube 81″ long on which 9 nozzles are fitted in line at 9″ spacings. Each tube is fitted with a separate ¾″ bore delivery hose, though ½″ bore is adequate for deliveries up to 6 gallons per minute for each gate. The gates can be adjusted up and down on the vertical pillar and their angle adjusted on the two hinge pins. If necessary, one or more nozzles can be blocked by fitting blank discs. These adjustments allow the gates to be adapted to various widths and heights of rows of bush plants.

The nozzles are of the ¼″ B.S.P. swirl type (1) fitted with 2-slot swirl plates 4/32 in. depth and 60-thousandths in. wide. The disc apertures are selected to give the required delivery at the selected tractor speed, the maximum being 18 nozzles with 8/64 discs at 75 lbs. per sq. in. flow pressure, which provides a delivery of approximately 17 gallons per minute.

Operation. The spray machine can be operated by medium-sized wheeled tractors of suitable track widths. A narrow-track tractor not exceeding 4 feet overall width is required for currant rows planted at 8 feet apart, and the wheels, especially the rear ones, must be fitted with simple guards. Unless the currant bushes are small, closer spacing of rows than 8 feet leads to extensive damage. Automatic spraying becomes most difficult, regardless of the width apart of the rows, just after picking, as the plants are spread into the alleys. The tractor must be fitted with a cab to protect the driver from spray drift, a simple hessian cover fitted over a steel tubular frame having been found adequate and preferable to a fully enclosed type.

Performance.

Blackcurrants. A comparison of lance and automatic spraying was made on 10-year-old well-grown currants planted in one plot at 8 ft. and in another plot at 10 ft. between rows, and 4 ft. between bushes.

The lance spraying was carried out by means of a tractor-trailer high-pressure spraying machine with a 300-gallon tank placed successively at points 150 ft. apart on the headland bordering the rows of currants. A hose 90 ft. long was used to spray 75 ft. along the row from each headland. One two-nozzle short-range broom with an output of 4 gallons per minute was used, one man handling the broom and two acting as hose pullers. Larger nozzle throughputs did not give better or faster results, as the limiting factor was the work associated with moving the hose over difficult soil conditions.

The automatic sprayer for comparison was towed between the rows at 3 m.p.h. and the number of nozzles and aperture size adjusted to give complete coverage under the prevailing wind condition. Six nozzles on each vertical gate with 8/64th in. disc apertures operating at 75 lbs. per sq. in. were found adequate for most conditions. All operations of filling the tank and spraying were undertaken by the tractor driver.

The spraying consisted of an application of a tar oil winter wash before bud-burst and of a combined lime sulphur and D.D.T. wash at the "grape" stage.

The volume of winter wash applied by hand spraying averaged 450 gallons per acre and the spraying required 35 man-hours and 11 tractor-hours inclusive per acre. These figures include an apportionment of all time included in the operation. This is mentioned as the non-spraying hours are often excluded from quoted figures on spraying. The overall cost, including labour, materials and overheads, was £12 per acre for the winter washing.

The automatic spraying was more economical of materials and considerably more economical of time. On an average, 300 gallons per acre were applied and this required only 3 man-hours and 3 tractor-hours inclusive. The overall cost for winter spraying was £4–10–0 per acre.

No significant difference was noted in the volume of wash applied to the bushes planted in the 8- and 10-foot rows, as the wider planting had produced large bushes.

Pyramid and cordon apples. A plantation of 5-year-old dwarf-pyramid and cordon apples, planted 8 feet between the rows and, in the case of the pyramids, approximately 4½–5 ft. high, were sprayed throughout the season with the automatic sprayer. The speed of the tractor was 2 m.p.h. and 9 nozzles fitted with 8/64th in. disc apertures on each vertical gate were used. The volume used to obtain complete coverage was 460 gallons per acre and the inclusive time was two hours. The complete operation was carried out by the tractor driver alone.

Summary.

(1) Automatic spraying of blackcurrants can be carried out satisfactorily at one-third of the inclusive cost of lance spraying. The method is equally suitable for dwarf-pyramid and cordon apples.

(2) A brief specification of the automatic sprayer is given.

(3) The automatic sprayer can be fitted with a simple gear pump with a throughput of 18–20 gallons per minute at 75 lbs. per sq. in. This gave a satisfactory performance.

Acknowledgments.

Many thanks are due to Messrs. C. H. Harper and J. H. Russell for carrying out the field spraying and keeping the records.

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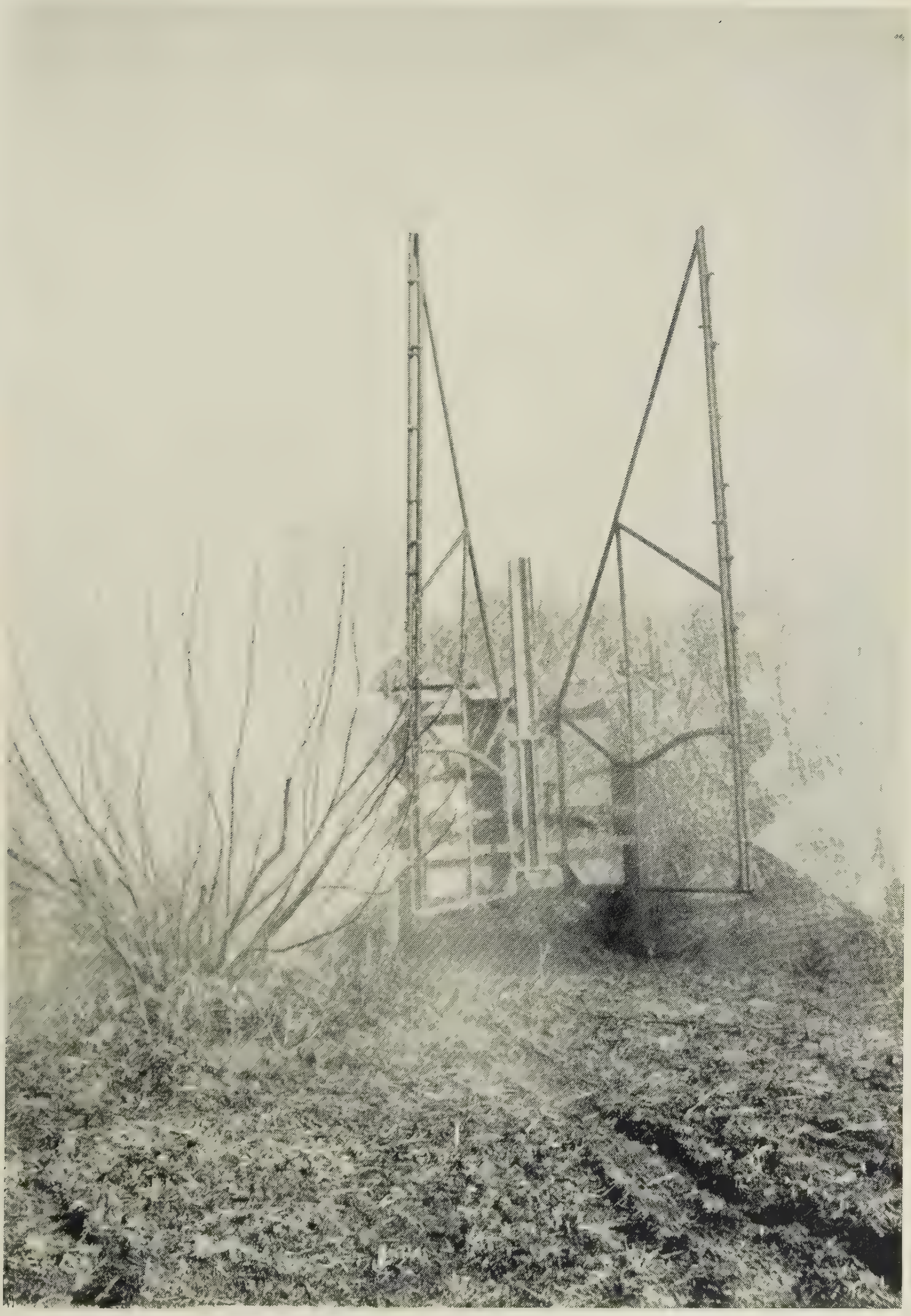
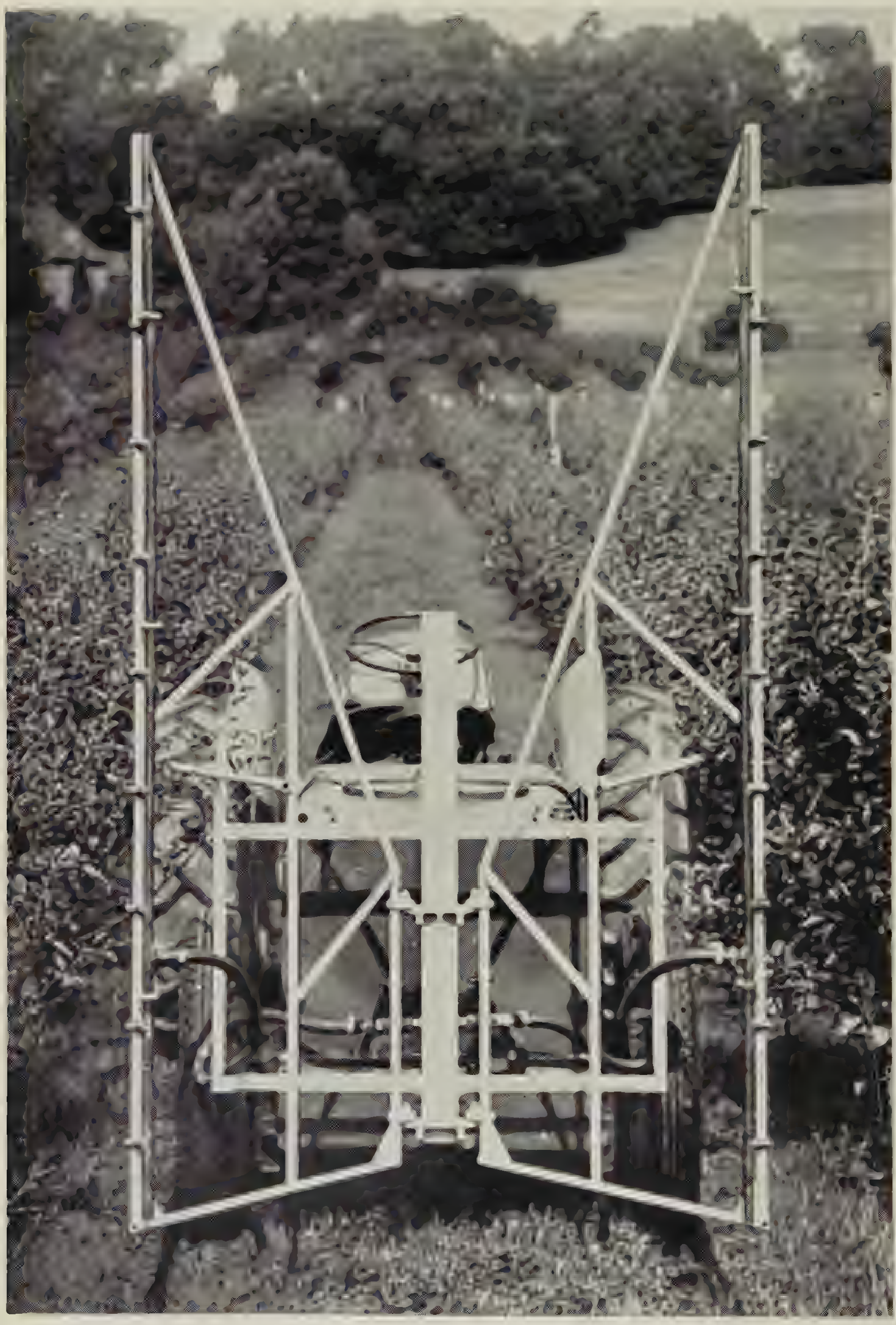
PLATE VII



Low pressure automatic spraying of blackcurrants.

PLATE VIII



Automatic sprayer showing the design of the adjustable spray gates.

SPRAY APPLICATION PROBLEMS:

VI. A LABORATORY TECHNIQUE USING *BOTRYTIS FABAE* ON BROAD BEAN FOR THE BIOLOGICAL EVALUATION OF FUNGICIDAL SPRAY DEPOSITS

By N. G. MORGAN.

The post-war development of small-volume spraying, both of insecticides and fungicides, has necessitated a reconsideration of some of the factors affecting the efficiency of methods of application. The large-volume spraying of a plant surface to the point of "run-off" of excess wash is assumed to achieve a complete protective film of fungicide deposit—although this is not always the case—while the application of small volumes may leave a discontinuous deposit scattered over the surface in a stippled pattern. It is important to establish the fungicidal effects that result from the different types of distribution of the spray deposit over the target. These variations in spray pattern are likely to affect not only immediate protective action but also leaching effects and liability to redistribution of the fungicide by rain, dew and other factors. The technique described in this paper may be used as a biological check to supplement chemical methods of analysis of amounts and distribution of the fungicide deposits.

If broad bean plants are sprayed with a dense suspension of spores of *Botrytis fabae* and kept under suitable conditions of temperature and humidity for several hours the lesions produced by the fungus appear as brown or black spots, the number depending upon the spore distribution on the leaf. Lesions will not appear if the spore suspension is in close proximity to a suitable fungicide deposit. A complete protectant "cover" of the deposit is indicated by total absence of lesions, and partial cover by clear areas, the size of which depends upon the distribution of the effective fungicide deposit, provided that the spore suspension and the method of applying it are standardised.

The technique, which, so far, has been used with copper suspensions only, is carried out in the following stages :—

(1) Bean plants or freshly detached leaves are exposed in the field or laboratory to the fungicide treatment to be investigated : the fungicide deposit is then allowed to dry.

(2) The plants or leaves are uniformly covered with the spore suspension and placed in moist chambers at 21° ± 2° C. for 16 hours to incubate the fungus.

(3) The spores germinate, and disease lesions are formed. The effect of the copper deposit on lesion distribution may then be examined and measurements made 16–24 hours after the time of inoculation.

Plant material.

The plants used are broad beans, var. Giant Windsor, raised singly in $3\frac{1}{2}$ inch pots in a cold greenhouse. They are most convenient to handle when about 4 weeks old, 4 inches high with 3 pairs of open leaves.

Spore material.

A sporing *Botrytis fabae* culture, on nutrient dextrose medium, 6–8 days after inoculation is shaken with a 1% sucrose solution and the resulting suspension diluted to give the required spore concentration. For routine work, convenient and sufficiently accurate counts of the number of spores in a drop of suspension on a slide under a coverslip can be made using the number seen, in one focal plane only, over a microscope field of known area. A convenient concentration was found to be 200 spores per 1·25 mm. diameter field. The suspension is used as soon as is practicable and not more than half an hour after making up.

Methods of applying the spore suspension.

The spore suspension may be applied through a spray tower or small paint spray gun in one of the two following ways depending upon the purpose for which the technique is being used :—

(*a*) The plant or leaf carrying a fungicide deposit is gently sprayed to run-off with the spore suspension and allowed to drain, leaving a complete film of suspension over the surface. This film is maintained throughout the spore germination period and any copper deposit present is therefore connected or " bridged " by water to the spores. The effective range of the deposit in preventing spore germination under these conditions can be seen by the resulting pattern of *Botrytis* lesions (Fig. 1*a*). This method requires the use of a wetting agent.

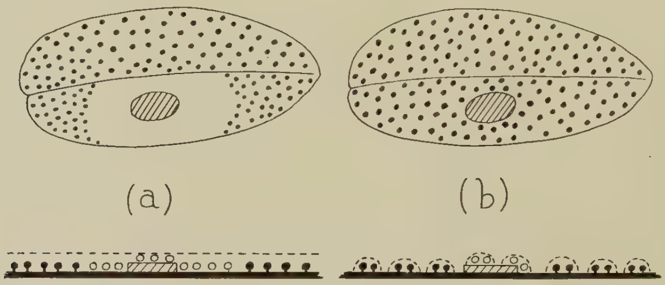


Fig. 1. Diagram of the effect of a fungicide deposit on a *Botrytis* spore suspension applied to the leaf : (*a*) as a continuous film ; (*b*) as separate droplets.

Most of the known commercial wetters were found to be fungicidal but the juice extracted from the pulped stems and leaves of broad bean plants with distilled water was found to be an effective non-fungicidal wetter. Ten 4-inch bean plants were sliced and pulped in a mortar with 25 ml. distilled water, strained through muslin and the liquor used to make up the spore suspension.

(*b*) Small droplets of the spore suspension are allowed to fall on the fungicide-treated plant surface from a fixed distance and time depending upon the design and performance of the spray nozzle. A uniform distribution of small closely-spaced droplets containing spores is obtained if care is taken to avoid overspraying and consequent coalescence of droplets. Since in this case the copper/spore water " bridges " are not continuous, only those spores in droplets falling on or touching the edge of an effective copper deposit will fail to produce lesions. The resulting clear areas therefore indicate the limits of the effective deposit (Fig. 1*b*).

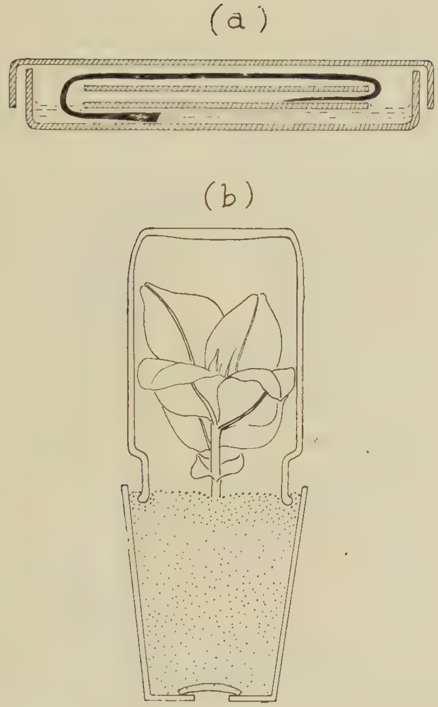


***Fig.* 2. Moist chambers for : (*a*) single bean leaves ; (*b*) bean plants.**

Method (*b*) probably more nearly represents the manner in which fungus spores arrive on the crop foliage under natural conditions. It is also possible that in method (*a*) some of the copper deposit is redistributed by the use of a wetting agent.

Spore incubation.

Immediately after application of the spore suspension each bean plant is covered by a 2 lb. jam-jar previously rinsed with warm water. The jar is lightly pressed into the pot soil forming a humidity chamber (Fig. 2*b*) and the whole placed in the constant temperature chamber. Complete bean plants in pots may therefore be exposed to copper fungicide spraying in the field and the technique used for biological analysis of the deposits obtained.

Single leaves may be individually treated throughout, or detached from treated plants for spore inoculation. Sufficient warm water is placed in a petri dish to cover the bottom and left until condensation appears on the inside of the lid. Each leaf is placed on a 3″ × 2″ glass plate with its distal end folded under and held in place by a second plate secured by a rubber band at each end. The assembly is then placed in the petri dish with the leaf petiole held in the water beneath the lower plate (Fig. 2*a*). The advantages of using single leaves are ease of handling and economy of incubator space, while the use of both jam-jars and petri dishes enable the experiments to be made without special humidity chambers and control apparatus. Single leaves can be treated with droplets of known size and copper concentration, applied singly through a hypodermic needle and micro-feed syringe and the effects of deposits arranged in pre-determined patterns obtained.

Plate IX illustrates leaves on which patterns of spray deposits have been placed by this method. The leaves were subsequently incubated after being sprayed to run-off with a *Botrytis* spore suspension. The photographs demonstrate that disease lesions occur densely on all parts of the leaf except in the areas around the spray deposits. Although the total volume of copper fungicide and the weight of copper, 0·0072 ml. and 0·018 mgm. respectively, were the same for each leaf, the size and distribution of the areas clear of lesions are shown to depend upon the number and distribution pattern of the fungicide deposits.

A serious limitation in the field use of the method is restriction to the broad bean plant, but it may be possible to develop similar techniques using other fungus diseases. A further difficulty is that the spore concentration in the suspension required to produce the necessary contrast between clear and diseased areas of the plant surface is far higher than that found under natural conditions. However, a deposit of copper fungicide which gives satisfactory protection from the heavy *Botrytis* spore suspension used in the laboratory can reasonably be expected to be satisfactory against any spore incidence occurring naturally in the field.

Summary.

A technique, using *Botrytis fabae* on broad bean, for the biological evaluation of copper fungicide deposits is described. Whole bean plants in pots or single detached leaves are treated with the fungicide and allowed to dry. They are then uniformly covered with a *Botrytis* spore suspension either as a complete film or as separate droplets, enclosed in individual glass humidity chambers, and placed in an incubator at 21° C. After 16 hours the distribution of the *Botrytis* lesion spots can be used as an indication of the distribution of an effective fungicide deposit.

The technique can be used :—

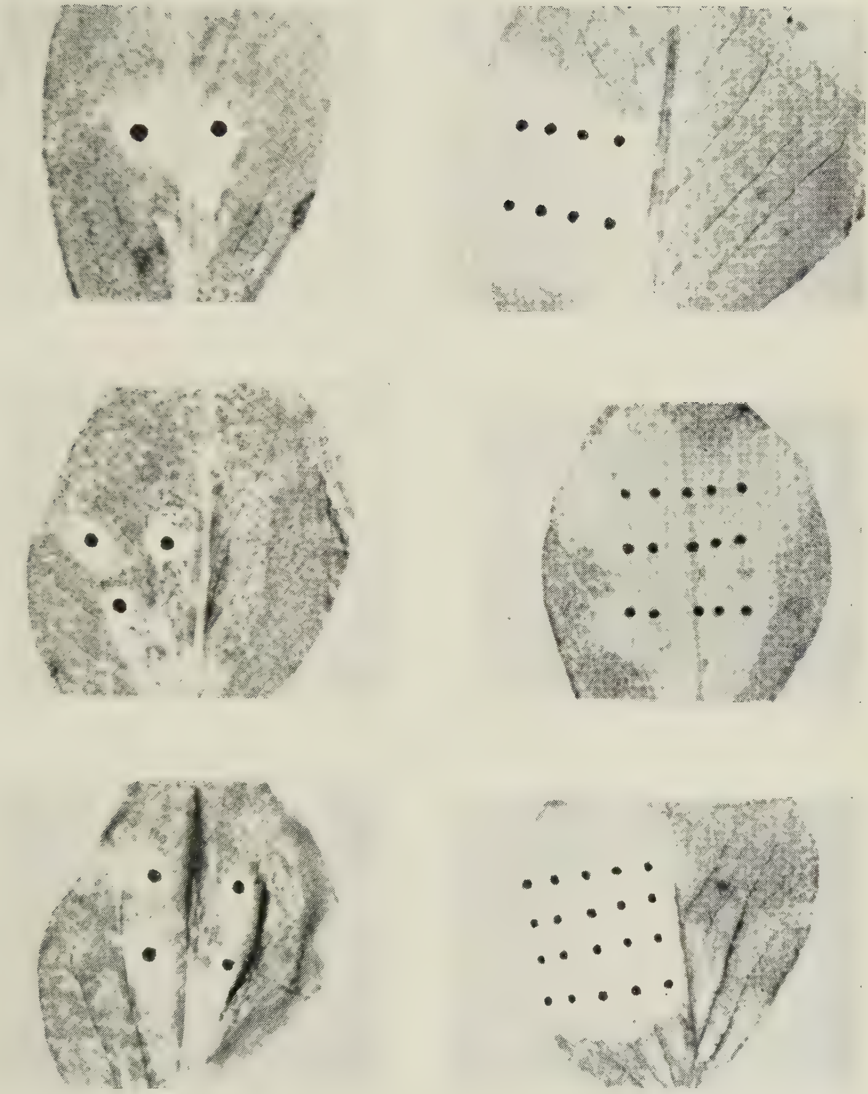
(a) as a biological check on chemical analysis and indicator methods.

(b) for investigating the factors influencing efficiency of fungicide deposits obtained by various methods of spray application.

Acknowledgments.

Thanks are due to Dr. Kearns and to the Mycology Department for invaluable help and advice, and to Mr. G. Bracey for much painstaking work on the technique.

PLATE IX.



Bean leaves infected by *Botrytis fabae*, showing healthy areas where infection has been inhibited by copper spray deposits. Note the effect of the pattern of distribution of the spots of fungicide. The total amount of fungicide on each leaf is the same.

SPRAYING EXPERIMENTS AGAINST APPLE AND PEAR SCAB AT LONG ASHTON, 1952.

By R. J. W. BYRDE and A. H. FIELDING.

The experiments described in this paper form a further continuation of those carried out in earlier years (Byrde and Marsh, 1951, 1952) on the fungicidal and phytotoxic effects of materials showing promise as possible substitutes for lime sulphur, which was used as a standard for comparison.

Plots.

The apple trials were made on Plot 23 in the Long Ashton Research Station plantations, on the varieties Cox's Orange Pippin, Worcester Pearmain, Lane's Prince Albert and Stirling Castle. The trees, planted in 1935, were in grass, regularly cut by a gang-mower, and were spaced at 21 ft.

The pears used were two 25-tree rows, of William's Bon Chrétien and Doyenne du Comice respectively, planted at 15 ft. in grass. The original trees were set out in 1937, but there were many younger replacements.

Spray materials.

The spray materials were formulated as follows :—

Lime sulphur, as the commercial concentrate, used at 2% pre-blossom and 1% post-blossom.

Phenyl mercury chloride, as a 2½% wettable powder on china clay, used at 2 lb. per 100 gal. to give a spray containing 0·0032% Hg.

Phenyl mercury acetate, as a wettable powder of 2% Hg content, used at 1·6 lb. per 100 gal. to give a spray containing 0·0032% Hg.

Diphenyl mercuric salt of di-2:2′-naphthylmethane-3:3′-disulphonic acid, as a water-miscible liquid containing 0·8% Hg, used at 3·2 pints per 100 gal. to give a spray containing 0·0032% Hg.

Heptadecyl glyoxalidine (341–SC), as a water-miscible liquid containing 34% active material, used at 3 pints per 100 gal. with 8 oz. hydrated lime added.

Trichloromethylthio-tetrahydrophthalimide (SR.406), as a 50% wettable powder used at 2 lb. per 100 gal.

The sprays were applied by means of a small mobile outfit with a single-nozzle broom (disc aperture 5/64 in. ; pressure 75 lb./sq. in.) or by a large power sprayer, incorporating a triple-nozzle broom (disc aperture 4/64 in. ; pressure 400 lb./sq. in.).

Lay-out of experiments and spraying dates.

Mercurials : Worcesters (Exp. A.10). The following five one-tree treatments were randomised in each of six blocks on Plot 23 : phenyl mercury acetate, phenyl mercury chloride, soluble organo-mercury sulphonate, lime sulphur and Control. Three applications were made, using the small spray outfit : pre-blossom on May 5th–8th and post-blossom on May 26th and June 25th. Applications of lime sulphur, phenyl mercury acetate and the soluble organo-mercury sulphonate on May 1st were immediately followed by a heavy thunderstorm, giving over 1 in. of rain, so this earlier application was disregarded.

Glyoxalidine : *Cox* (Exp. A.9). The lay-out of the block of thirty trees on Plot 23 used for trials of glyoxalidine sprays in 1950 (using 341–C mixed glyoxalidines) and 1951 (using heptadecyl glyoxalidine) was unchanged. Five equal strips were treated respectively, from east to west, with heptadecyl glyoxalidine, no spray, lime sulphur, no spray and heptadecyl glyoxalidine. Sprays were applied by means of the large sprayer on April 28th (pre-blossom) and on May 26th and July 1st (post-blossom).

Phthalimide : *Cox and Worcester* (Exp. A.7). The following three ten-tree treatments were randomised in each of two blocks on Plot 23 : phthalimide spray, lime sulphur and Control. Adjacent "guard" rows of Worcester trees received corresponding treatment. Sprays were applied by means of the larger sprayer on April 28th (pre-blossom) and on May 26th and June 25th (post-blossom).

Phytotoxicity : *Lane and Stirling* (Exp. A.11). Nine trees of each variety were allocated as follows :—

Controls, 3 trees ; phthalimide spray, 3 trees ; phenyl mercury chloride, 3 trees.

The dates of application and the sprayer used for the mercurial and the phthalimide fungicides were those listed above under Experiments A.10 and A.7 respectively.

Pear scab trial : *Doyenne du Comice and Williams B.C.* (Exp. P.2). Each variety was divided into four 6-tree blocks within which the following three treatments were randomised on single trees : heptadecyl glyoxalidine, phthalimide and no spray. Sprays were applied by means of the small sprayer on April 15th (pre-blossom) and on May 17th and June 16th (post-blossom).

All apple plots received a separate petal-fall application of nicotine and wetting agent prior to the fungicidal post-blossom spray, for the control of apple sawfly.

Notes on weather and method of recording.

A description of the weather of the summer of 1952 is given by Kearns, Marsh and Morgan (1953) elsewhere in this Annual Report.

Mean percentage scab areas per leaf and per fruit were assessed by Tehon and Stout's method as amplified by Marsh (1947). The samples taken comprised at least 250 leaves, or 100 fruits, per tree, picked from a cone-shaped space extending to the centre of the tree from the north side. For the assessment of scab on pear fruits, where infection occurred as numerous small spots scattered over the fruit surface, models were prepared showing 0·5, 1, 2, 5, 10 and 20% infection of the total area (see Croxall *et al.*, 1953).

Experimental results.

Mercurial trial : *Worcesters* (Exp. A.10). Mean percentage areas scabbed were assessed on leaves on July 25th–29th and on fruit on Aug. 18th–21st. Results are assembled in Table I.

Glyoxalidine trial : *Cox* (Exp. A.9). Leaf assessments were made on July 31st and fruit assessments on Sept. 8th–9th. Results are summarised in Table II.

For both leaves and fruits, the two treatments are significantly better than Control, but do not differ significantly from each other.

TABLE I.

RESULTS OF MERCURIAL TRIAL : WORCESTERS.
(Sprays applied May 5th–8th, May 26th, June 25th).

Spray material	Concn.	Mean % area scabbed	
		Leaves	Fruits
Phenyl mercury acetate	0·0032% Hg	0·24	0·04
Phenyl mercury chloride	0·0032% Hg	0·61	0·19
Soluble organo-mercury sulphonate ..	0·0032% Hg	1·89	0·68
Lime sulphur	2% then 1%	0·27	0·01
Control	—	1·56	1·16
Least sig. diff. at $P = 0{\cdot}05$		0·47	0·38

TABLE II.

RESULTS OF GLYOXALIDINE TRIAL : COX.
(Sprays applied April 28th, May 26th and July 1st).

Spray material	Concn.	Mean % area scabbed	
		Leaves	Fruits
Heptadecyl glyoxalidine (34%) ..	0·375%	0·04	0·12
Lime sulphur	2% then 1%	0·03	0·09
Control	—	0·61	3·39

Phthalimide trial : *Cox and Worcester* (Exp. A.7). Leaf assessment was carried out on Worcesters only (July 24th) : fruit assessments were made on August 14th (Worcester) and September 10th–13th (Cox). Table III summarises the results obtained.

TABLE III.

RESULTS OF PHTHALIMIDE TRIAL : WORCESTER AND COX.
(Sprays applied April 28th, May 26th and June 25th).

Spray material	Concn.	Mean % area scabbed		
		Worcester		Cox
		Leaf	Fruit	Fruit
Phthalimide spray (50%) ..	0·2%	0·09	0·00	0·08
Lime sulphur	2% then 1%	0·16	0·00	0·09
Control	—	2·18	0·46	1·83
Least sig. diff. at $P = 0{\cdot}05$		0·96	0·25	0·28

Apples : phytotoxicity.

As in previous years (Byrde and Marsh, 1951, 1952) phytotoxicity was assessed numerically by fruit counts on August 21st (Stirlings) and September 5th (Lanes). The results of the 1952 trials are given in Table IV.

TABLE IV.

MEAN NUMBERS OF FRUIT PER TREE FOLLOWING FUNGICIDE TREATMENTS : STIRLING AND LANE.

Treatment	Concn.	Mean no. fruit per tree	
		Stirling	Lane
Phthalimide spray (50%)	0·2%	186	152
Phenyl mercury chloride (2·5%) ..	0·2%	185	153
Control	—	135	158

The Cox's Orange Pippin crop in the glyoxalidine and phthalimide trials (Exps. A.9 and A.7 respectively) was too large for convenient estimation of total numbers of fruit. Instead, the number of orchard boxes (40 lb.) per tree was recorded. (Table V).

TABLE V.

MEAN CROP OF FRUIT PER TREE FOLLOWING FUNGICIDE TREATMENT : COX.

Treatment	Concn.	Crop (Orchard boxes)	
		Phthalimide trial	Glyoxalidine trial
Phthalimide spray (50%)	0·2%	3·23	—
Heptadecyl glyoxalidine (34%) ..	0·375%	—	2·25
Lime sulphur	2% then 1%	2·54	1·41
Control	—	1·40	1·67
Least sig. diff. ($P = 0{\cdot}05$)		0·64	0·83

The reduced figures for the fruit crop on lime sulphur and Control plots in both these experiments were associated with marked defoliation from July onwards. While that on the lime sulphur plots was almost certainly due to normal sulphur scorch, that on the unsprayed plots was ascribed to a severe early attack of apple scab on the current season's wood, leading to desiccation during the hot dry weather of July.

Effect of scab fungicides on apple mildew.

An attack of apple mildew (caused by *Podosphaera leucotricha* (Ell. and Everh.) Salm.) developed on Cox trees in the glyoxalidine trial (Exp. A.9) in which the layout corresponded with that in 1950 and 1951. On July 31st, fifty shoots of the current season's growth were examined on each tree, and the number showing apple mildew infections was recorded. Table VI summarises the results obtained.

TABLE VI.

EFFECT OF SCAB FUNGICIDES ON INCIDENCE OF APPLE MILDEW.

Treatment for past three seasons	Concn.	Mean % shoots infected in July, 1952
Heptadecyl glyoxalidine (34%)* ..	0·375%*	36·8
Lime sulphur	2% pre-blossom 1% post-blossom	15·0
Control	—	42·0

*In 1950, mixed glyoxalidines (341–C), containing 50% active materials, were used at 0·25%.

The figure for lime sulphur is significantly lower than that for each of the other two treatments, which do not differ significantly from each other.

Pears : scab control (*Williams*).

An estimate of scab on the fruit, while still on the tree, was carried out on July 21st : it was feared that severe fruit drop might occur later in the season on the heavily infected Controls, and render impossible an accurate estimate at picking time. This in fact occurred. Results are presented in Table VII.

TABLE VII.

RESULTS OF TRIAL ON WILLIAMS PEARS.

(Sprays applied April 15th, May 17th and June 16th).

Treatment	Concn.	% fruit area scabbed
Phthalimide spray (50%) ..	0·2%	1·59
Heptadecyl glyoxalidine (34%) ..	0·375%	1·36
Control	—	3·57

Largely owing to a high tree-to-tree variability in the results, there is no significant difference between these figures.

Pears : phytotoxicity (*Comice*).

The total crop number per tree was estimated on Sept. 4th. Results are presented in Table VIII.

The figure for glyoxalidine is just significantly greater than that for Control.

TABLE VIII.

MEAN NUMBERS OF PEARS PER TREE FOLLOWING FUNGICIDE TREATMENT : DOYENNE DU COMICE.

Treatment	Concn.	Mean no. fruits per tree
Phthalimide spray (50%) ..	0·2%	61
Heptadecyl glyoxalidine (34%) ..	0·375%	80
Control	—	48

Discussion.

The 1952 season began with two periods of heavy rainfall—early May and early June—which were particularly favourable to apple scab development. Subsequent dry weather in late June and July checked the disease, but the figures quoted show that leaf and fruit infections on the Controls were moderately heavy. A severe attack of scab on wood of the current season on unsprayed trees of the variety Cox's Orange Pippin led to severe defoliation and fruit drop.

In 1951, three mercurial sprays, tested at the comparatively low concentration of 0·002% Hg, gave approximately equal performance against apple scab, but all were inferior to lime sulphur (Byrde and Marsh, 1952). In 1952, however, the scab control at a level of 0·0032% Hg was more variable. While the phenyl mercury acetate and phenyl mercury chloride formulations gave figures comparable with those for lime sulphur, the organo-mercury sulphonate was inferior, both to lime sulphur in 1952, and to its own performance at the lower concentration in 1951. There was a considerable tree-to-tree variability in scab level in this plot in 1952, resulting in less satisfactory discrimination between the treatments. The variability may well be due to the repeated use of this plot for scab experiments over a number of years.

The fungicidal and phytotoxic properties of heptadecyl glyoxalidine were again compared with those of lime sulphur on Cox's Orange Pippin and, as in the past two seasons, scab control with the two materials was approximately equal. In 1952 the crop from the trees sprayed with heptadecyl glyoxalidine was just significantly greater than that from those receiving lime sulphur.

The phthalimide spray has maintained its promise as a non-phytotoxic scab spray of high fungicidal value. It has again shown itself at least the equal of lime sulphur against apple scab on Worcester Pearmain, and also on Cox's Orange Pippin. Furthermore, the crop on trees of the latter variety sprayed with the phthalimide was significantly greater than that on trees sprayed with lime sulphur (where sulphur scorch occurred), and on those left unsprayed (where the severe twig infection led to fruit drop).

Little information is at present available on the performance of the newer organic fungicides against apple mildew. The data summarised in Table VI are therefore of particular interest : they illustrate the marked build-up of mildew which has followed the substitution of glyoxalidines for lime sulphur for three consecutive years in a part of England where the disease is not normally a serious problem. Data on the effect of the phthalimide spray on apple mildew at Long Ashton are not yet available.

The number of spray applications used in these experiments—three only—calls for comment. While at least four applications are used by most commercial growers, it is felt that for experimental purposes a better discrimination between fungicides follows the use of more widely spaced applications. Under such conditions, relatively weak fungicides are unable to afford a good control of the disease, while the more potent ones still give adequate control.

Summary.

On Worcester Pearmain apples at Long Ashton in 1952, the level of scab control obtained by three applications of lime sulphur was approximately equalled by that following three applications of phenyl mercury acetate and phenyl mercury chloride at 0·0032% Hg. An organo-mercury sulphonate material at the same concentration of mercury proved relatively ineffective.

Trichloromethylthio-tetrahydrophthalimide (0·2% of a 50% preparation) proved the equal of lime sulphur in scab control on Worcester Pearmain and Cox's Orange Pippin apples, and resulted in a significantly greater crop per tree on the latter variety. In another experiment on Cox's Orange Pippin, similar results were obtained from the use of heptadecyl glyoxalidine (0·375% of a 34% preparation). There was significantly less apple mildew (caused by *Podosphaera leucotricha*) on plots which had received lime sulphur during the past three years than on those where it had been omitted or replaced by glyoxalidine sprays.

No loss of crop followed the use of phenyl mercury chloride or the phthalimide spray on Lane's Prince Albert and Stirling Castle apples, or that of heptadecyl glyoxalidine or the phthalimide on Doyenne du Comice pears.

Acknowledgments.

The authors wish to thank Mr. R. W. Marsh for his interest in the trials. They also gratefully acknowledge the help of Mr. C. W. Harper (power spray operator) and of Dr. E. Williams, Miss R. Crowe, Mr. H. Terry, Miss M. Marlow and Miss P. Tozer (scab recorders).

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THE CONTROL OF FERMENTATION IN CIDER. II. FURTHER OBSERVATIONS ON THE INFLUENCE OF NITROGEN AND ACCESSORY GROWTH FACTORS ON THE STABILITY OF CENTRIFUGED CIDERS.

By L. F. BURROUGHS.

The Bramley's Seedling juices examined in previous years (1, 2) in the investigation of the control of fermentation of ciders, have been of medium or high nitrogen content. The course of utilisation of nitrogen in these juices was examined in detail and used as an indication of the growth and distribution of the yeast during fermentation. The information gained in this way has given a rational explanation of the principles involved in the efficient use of the centrifuge for controlling fermentation.

The stability of a cider after centrifuging was shown to depend on the extent of yeast growth which had previously taken place ; where yeast growth had gone to completion the cider was relatively stable. In the Bramley's Seedling cider examined in 1950–51, the stability of the centrifuged cider was found to be due to a combined deficiency of both nitrogen and aneurin, which prevented further growth of yeast.

In the present paper, the investigation has been continued on the same general lines but with the object of seeing if the previously discovered principles are applicable to a wider range of juices. The work is divided into two main sections : (a) the course of yeast growth during the undisturbed fermentation and the stability of the ciders after centrifuging have been examined in detail for the variety Stoke Red and in less detail for four other varieties ; (b) the nutritional deficiencies responsible for the stability of the cider after centrifuging have been examined for all five varieties.

Experimental.

Cider-making Procedure, and Sampling Technique.

Stoke Red. Approximately 4 tons of fruit were washed, milled and pressed on 10/11/51, the juice was bulked and thoroughly mixed in a slate vat before being distributed uniformly into a series of hogsheads. These were situated in the Cider-house and fermentation was allowed to proceed normally.

The juice in Cask 1 was allowed to ferment to dryness undisturbed and the cider was left on its lees. The contents of the other casks were centrifuged at different specific gravities using a Sharples centrifuge (16,000 r.p.m. and 80 gal./hour) and were subsequently stored in smaller hogsheads for observation of their stabilities. Samples were removed from Cask 1 at frequent intervals during the primary fermentation ; to prevent acetification due to access of air, the cask was topped-up occasionally with fermenting juice which had been reserved for this purpose. Samples were also taken from the casks of centrifuged cider at suitable intervals during storage. To prevent access of air, these casks were fitted with permanent syphons and the cider covered with a layer of liquid paraffin (B.P.). The same protection was used on Cask 1 when the supply of fermenting juice for topping-up was exhausted.

Other Varieties—Kingston Black, Yarlington Mill, Bramley's Seedling, Tardive Forestier. Only one hogshead of juice of each of these varieties was available. For each variety, the fermenting juice was centrifuged at a specific gravity in the region of 1,025 and the cider stored in another hogshead for observation of its stability. Samples were taken from these casks, both during the primary fermentation and during storage, the casks of centrifuged cider being protected from acetification by a layer of liquid paraffin.

Analytical Methods.

Specific gravity was determined at 20° C. using specific gravity bottles and weighing against a counterpoise (3).

The methods for total- and soluble-nitrogen and phosphorus were described in the previous paper (2).

The design of the experiments to determine the nutritional deficiencies preventing further yeast growth in the centrifuged ciders was slightly different from that previously described (2). The yeast nutrients added are shown in Table II and consisted of twelve different combinations of nitrogen, minerals, aneurin and other growth factors, each combination being in triplicate. The cider, together with the required addition of nutrients, was incubated at 25° C. in 4 oz. medicine bottles each closed with a fermentation lock, and the effect of the added nutrients was measured in terms of fermentation (loss of specific gravity) and yeast growth.

The experiments were set up aseptically : all bottles, flasks, fermentation locks and the liquid paraffin were sterilised for one hour at 150° C. ; rubber bungs for fermentation locks were sterilised in 60% alcohol ; the syphon tube and inorganic nutrient solutions were autoclaved ; pipettes were sterilised chemically and rinsed with fresh distilled water ; growth factor solutions were not sterilised but were kept under toluene in the refrigerator, the toluene being removed by a stream of filtered air before use.

The cider under test (4·5 litres) was divided into three parts ; to one of these nitrogen (10 mg.N/100 ml.) was added as NH_4Cl solution, to another a composite solution containing nitrogen and minerals[*], and to the third no addition was made. Each of these ciders was syphoned into a series of twelve bottles containing the appropriate growth factor[+] additions in the form of 0·5 ml. of solution. The surface of the cider in each bottle was then covered by a few drops of liquid paraffin and the bottle closed with a fermentation lock in a rubber bung. The lock was sealed with mercury and each bottle was weighed. All bottles were then incubated at 25° C. and weighed at intervals to follow the progress of the fermentation. The experiment was terminated when the loss in weight showed that certain of the treatments were approaching dryness.

At the end of the experiment the bottles were opened, the liquid paraffin removed and the contents thoroughly mixed to re-suspend the yeast and

[*] Mineral nutrients consisted of *Phosphorus* as KH_2PO_4, *Magnesium* as $MgCl_2$, *Calcium* as $CaCl_2$, *Sulphur* as K_2SO_4, each element being added at the rate of 5 mg./100 ml., and *Micro-nutrients* iron, manganese, zinc and molybdenum each at 1 p.p.m. and copper at 0·1 p.p.m.

[+] *Growth factors* were added at the rate recommended for yeast growth by Hopkins and Pennington (4), namely, aneurin 0·25 μg./ml., inositol 25 μg./ml., Ca pantothenate and nicotinic acid each at 2·5 μg./ml., pyridoxin 1·0 μg./ml., *p.* aminobenzoic acid 0·25 μg./ml. and biotin 0·0075 μg./ml.

de-gassed to remove dissolved CO_2. The specific gravity of the cider was determined. The amount of yeast present in the cider was determined turbidimetrically on a Spekker Absorptiometer using orange filters (Ilford 607). The instrument was set to a maximum drum-reading on a *filtered* portion of cider from one of the experimental treatments. The use of orange filters largely eliminated absorption due to colour of the cider and standard curves of suspensions of fresh, pressed baker's yeast in different filtered ciders showed fairly close agreement.

In all these experiments, the extent of fermentation was measured as decrease in specific gravity (degrees) and yeast growth as increase in turbidity expressed as mg. moist yeast per 100 ml. cider.

Results.

Details of the composition of the juices of the five varieties are given in Table I.

TABLE I.

COMPOSITION OF THE FRESHLY PRESSED JUICES.

Variety		Stoke Red	Kingston Black	Yarlington Mill	Tardive Forestier	Bramley's Seedling
Specific Gravity.. ..		1,046·0	1,050·1	1,044·4	1,042·2	1,043·4
Titratable Acidity .. (g.malic acid/100 ml.)		0·60	0·58	0·28	0·22	0·91
pH		3·32	3·53	3·90	3·87	3·01
Tannin* (g./100 ml.) ..		0·25	0·28	0·25	0·30	0·10
Rate of Fermentation+..		0.4	0·6	1·8	3·5	3·9
Nitrogen (mg.N/ 100 ml.)	Total ..	2·38	2·43	3·68	8·22	8·62
	Soluble ..	2·24	2·31	3·35	7·86	8·00
Phosphorus (mg.P/ 100 ml.)	Total ..	7·65	9·80	3·58	6·58	5·81
	Soluble ..	7·44	9·80	3·58	6·52	5·73

* Tannin is estimated as $KMn0_4$ reducing substances and is expressed as gallotannin (g./100 ml.).

+ Rate of Fermentation corresponds to degrees of S.G. lost per day at 25° C.

The acid and tannin contents of these juices were within the normal ranges for the varieties. Stoke Red and Kingston Black are typically slow-fermenting varieties ; the two examples here were outstanding in this respect and in their low nitrogen contents. The Bramley's Seedling juice was very similar to that examined in the previous year (2) in its rate of fermentation, nitrogen and phosphorus contents. The rates of fermentation of these five juices show a close relation with their nitrogen contents and are independent of their phosphorus contents.

The Utilisation of Nitrogen during Yeast Growth and Fermentation and the Stability of the Centrifuged Ciders.

Stoke Red. The changes in total- and soluble-nitrogen and in specific gravity during the undisturbed fermentation of the juice in Cask 1 are shown in the graph (Fig. 1). The interpretation of the nitrogen data into terms of the growth and distribution of yeast (in the lees and in suspension) has been given in previous papers (1). For the sake of clarity, curves of yeast growth are omitted from the graphs.

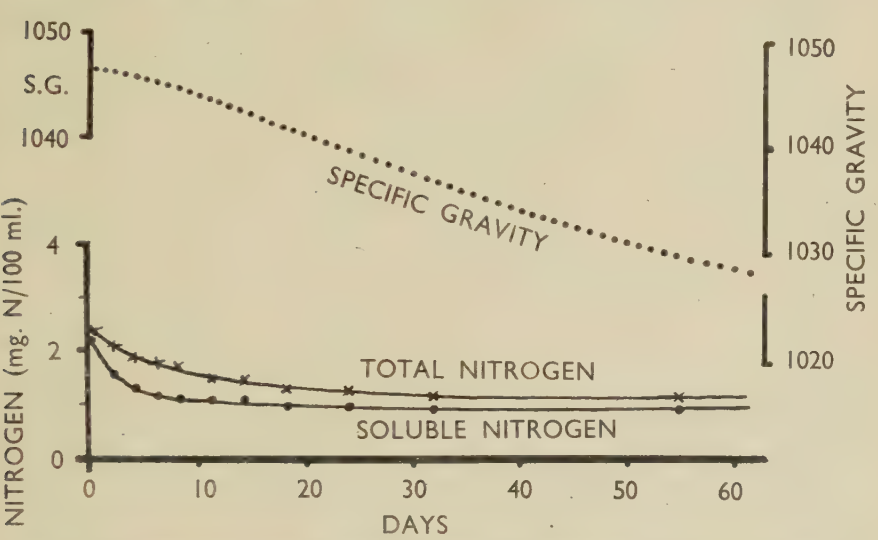

Fig. 1. Utilisation of nitrogen during the undisturbed fermentation (Stoke Red, Cask 1).

This juice was characterised by the very small amount of yeast growth which took place and by the extremely slow fermentation. After 21 weeks the specific gravity was still 1,015 and at 35 weeks was 1,004. The soluble nitrogen at this time was 0·76 mg.N/100 ml. so that the amount of nitrogen utilised during the whole fermentation was only 1·48 mg.N/100 ml. of which approximately 1 mg. was taken up during the first five days. With this very small amount of yeast growth, no increase in soluble nitrogen at the end of the fermentation, due to autolysis, was detected.

Replicate casks of the same juice were centrifuged at different specific gravities during their fermentation. Certain small differences between the rates of fermentation in the different casks were observed ; in particular, the juice in Cask 1 fermented slightly faster than that in most other casks. This may have been due to the unavoidable disturbance of taking frequent samples from Cask 1 and to the slight penetration of air through the layer of liquid paraffin.

The specific gravities at which the ciders in the various casks were centrifuged, and the subsequent changes in specific gravity during storage are shown in Fig. 2 together with the specific gravity curve for the undisturbed fermentation in Cask 1.

All these ciders were relatively stable after centrifuging ; the slightly accelerated fall in specific gravity between the 150th and 245th days may be

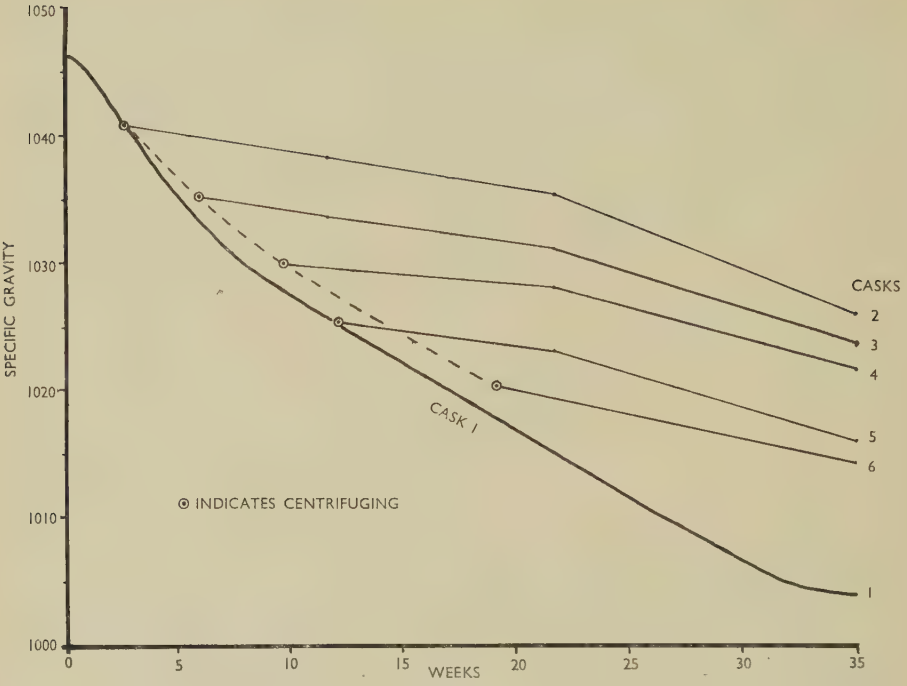

Fig. 2. Changes in specific gravity during the undisturbed fermentation (Cask 1) and of centrifuged ciders during storage (Casks 2–6).

attributed to the higher temperatures during the months April to July. Although the cider in Cask 2 had fermented only 5½ degrees of specific gravity when it was centrifuged, the yeast growth which occurred prior to centrifuging had reduced its soluble nitrogen content from 2·24 to 1·17 mg.N/100 ml. Further growth of yeast during storage of the centrifuged cider was therefore very limited, and the soluble nitrogen on the 245th day was 0·81 mg.N/100 ml. being a decrease of 0·36 mg.N/100 ml. in 226 days. In the other casks of centrifuged cider, the decreases in soluble nitrogen during storage were even less than this.

Since the extent of yeast growth which this Stoke Red juice was able to support was so limited, the changes in soluble phosphorus content of the fermenting juice were very small and are not reported here.

Other varieties—Kingston Black, Yarlington Mill, Tardive Forestier and Bramley's Seedling. For these varieties the changes in total- and soluble-nitrogen and in specific gravity during the primary fermentations are shown in Figs. 3 to 6. Since only one cask of each variety was available, the subsequent changes in specific gravity during storage of the centrifuged ciders are given in tabular form with each graph. For comparative purposes, the same scale is used for each variety, except for Kingston Black where the time scale is compressed on account of the slow fermentation. The Kingston Black cider was centrifuged at a specific gravity of 1,032·3 and the other varieties between 1,021 and 1,025. In all varieties, yeast growth had gone to completion at the time of centrifuging and all varieties were fairly stable for six to eight weeks after centrifuging. The Kingston Black cider maintained its specific gravity well throughout the summer but the other varieties were much less stable during this time.

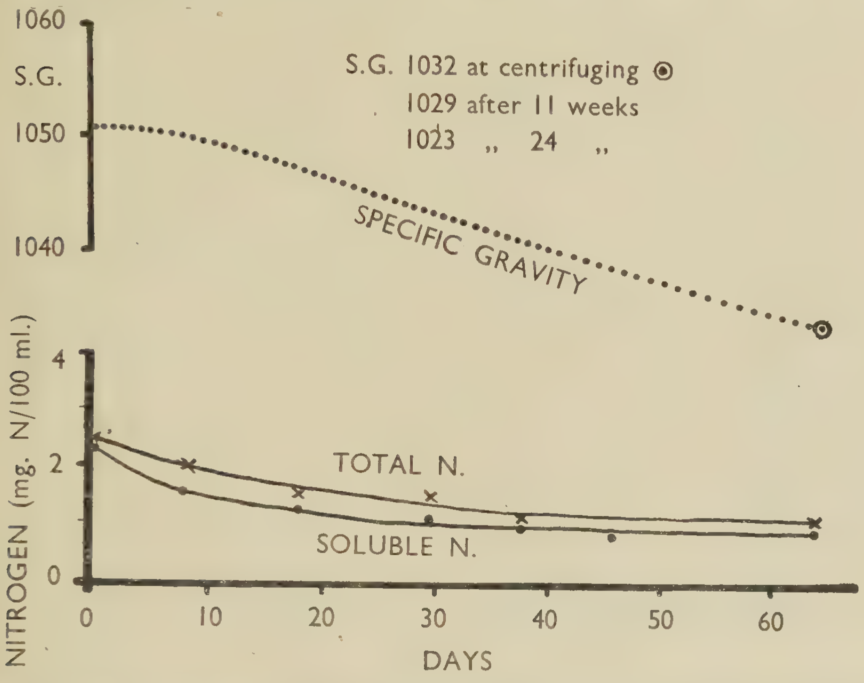

Fig. 3. Utilisation of nitrogen during fermentation prior to centrifuging (KINGSTON BLACK).

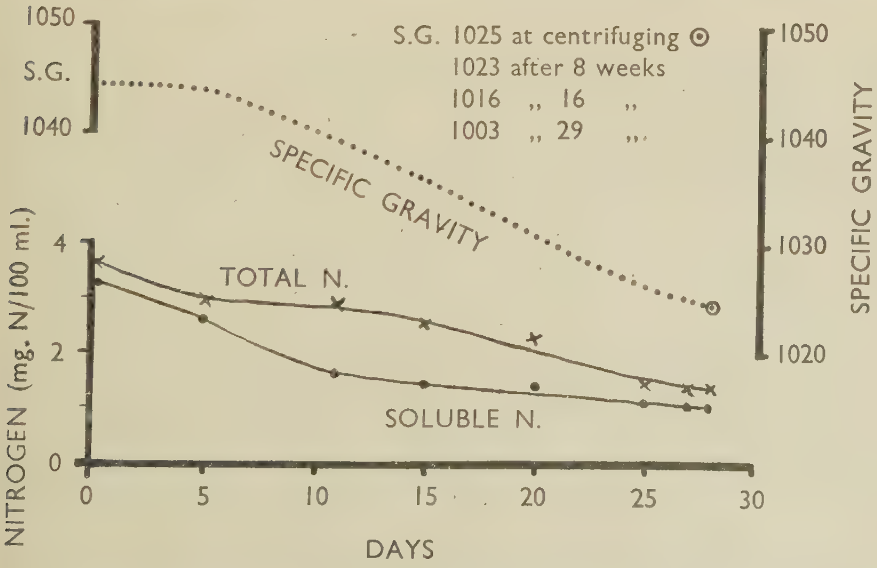

Fig. 4. Utilisation of nitrogen during fermentation prior to centrifuging (YARLINGTON MILL).

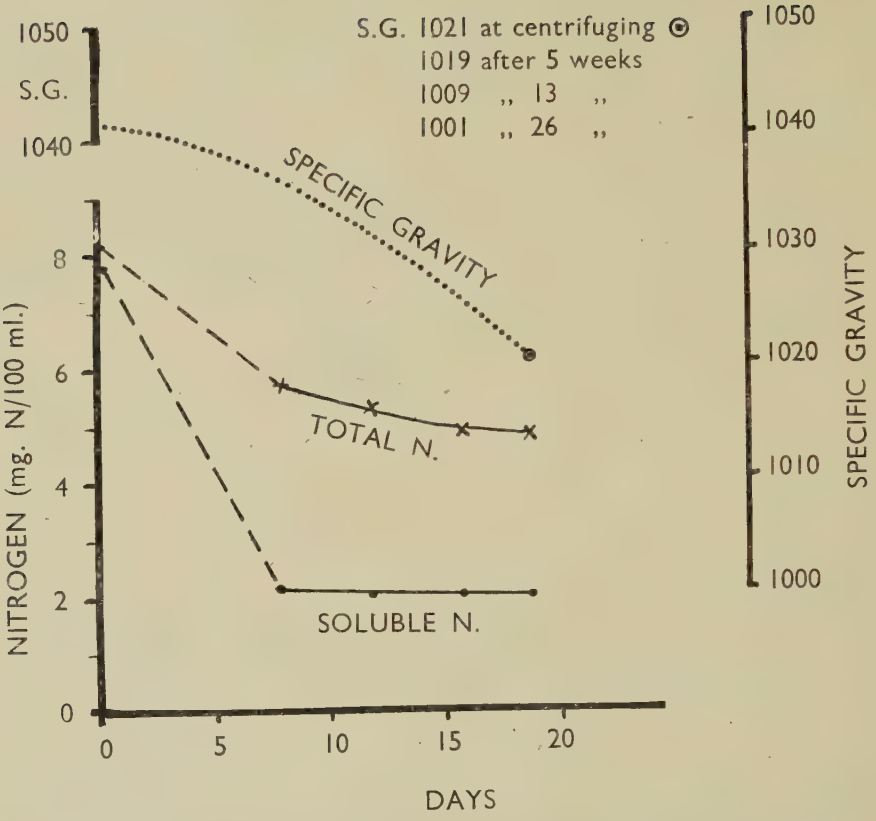

Fig. 5. Utilisation of nitrogen during fermentation prior to centrifuging (TARDIVE FORESTIER).

Nutritional Deficiencies responsible for the Stability of the Centrifuged Ciders.

In the previous paper in this series, it was shown that the stability of a centrifuged Bramley's Seedling cider was due to a combined deficiency of both nitrogen and aneurin. One of the main objects of the present investigation was to ascertain if the stability of these ciders also was due to the same deficiencies. The design of the experiments in which different yeast nutrients were added to the centrifuged ciders was described in an earlier section.

In spite of care in setting up the experiments, there was considerable variation between triplicates, a variation which was greatest in those treatments giving a large response. The effects of this variation are allowed for statistically in the calculation of the significant difference between means.

The results of the experiment on Cask 2 of Stoke Red are tabulated in full (Table II) together with means of triplicates expressed as percentages of the treatment giving maximum response. The latter method, although using derived data, gives a clearer presentation of the relative magnitudes of the results.

In this experiment the results are clearly divided into three groups of increasing magnitude of response :—

Group A, all treatments omitting nitrogen—a very small response.

Group B, all treatments containing nitrogen but omitting aneurin—a medium response.

Group C, all treatments containing both nitrogen and aneurin —a large response.

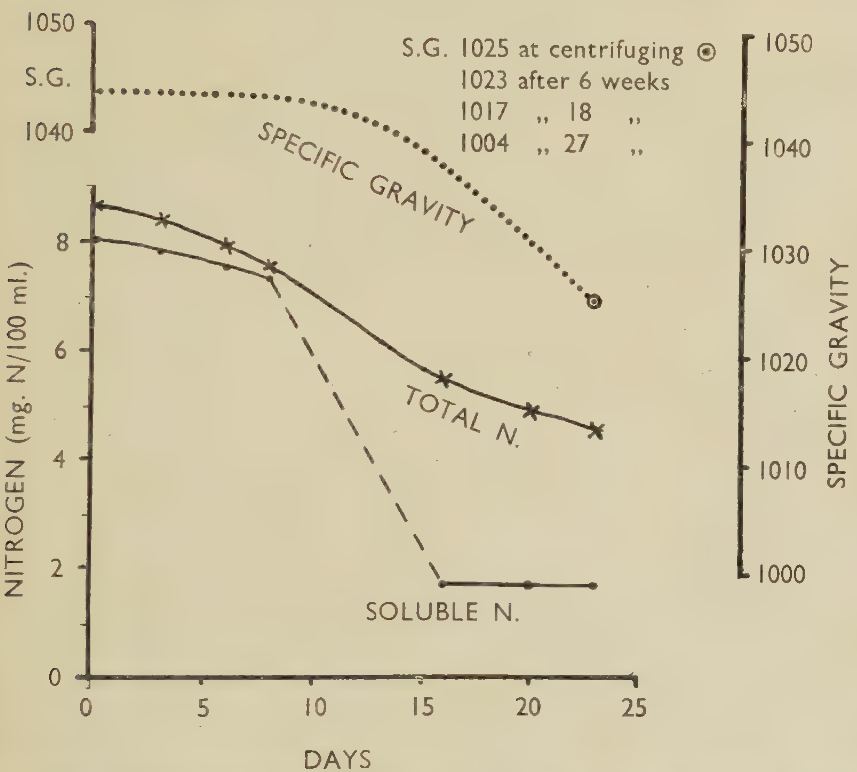

Fig. 6. Utilisation of nitrogen during fermentation prior to centrifuging (BRAMLEY'S SEEDLING).

Differences in response between these groups A, B and C are obvious and do not require statistical proof. These differences constituted the main findings of the experiment, namely, that the stability of the centrifuged cider was due to a combined deficiency of both nitrogen and aneurin.

Further information can be gained by consideration of the results of different treatments *within* each of the groups A, B and C. These were examined statistically (analysis of variance) and the differences required for significance* between means of triplicates, expressed as percentages of the maximum, are given in Table III. Thus it is seen that in group B, in the absence of aneurin the other growth factors had no effect, whereas in the presence of aneurin (group C) the supplementary effect of the other growth factors was significant. Other conclusions to be drawn from this experiment on Stoke Red cider and from similar experiments on ciders of the other four varieties are discussed later.

*Significant difference between means of triplicates = $\sqrt{\frac{\text{Error Mean Square}}{3}} \times \text{'t} \sqrt{2}.$

TABLE II.

STOKE RED (CASK 2).

Cider centrifuged on 27/11/51 at specific gravity 1,040·6.
Growth Factor Experiment :—
Date commenced 30/11/51 ; duration 6 days.
Initial specific gravity 1,040·6.
Initial yeast content 7 mg./100 ml.

Added Nitrogen or Minerals	Added Growth Factors							
	Nil		Six growth factors†		Aneurin		Aneurin plus six growth factors†	
	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*
Nil ..	1·3	*28*	1·1	*20*	1·1	*21*	1·0	*23*
	1·1	*26*	1·1	*20*	1·3	*26*	1·1	*21*
	1·0	*24*	1·0	*21*	0·9	*23*	0·9	*20*
Nitrogen ..	7·2	*150*	6·4	*139*	22·0	*503*	28·1	*463*
	6·6	*125*	6·0	*120*	22·3	*453*	28·4	*523*
	6·4	*113*	6·5	*115*	20·8	*373*	29·3	*653*
Nitrogen plus Minerals ..	6·3	*125*	7·0	*166*	15·4	*303*	29·3	*568*
	6·4	*110*	6·4	*145*	15·1	*266*	24·5	*393*
	6·6	*123*	5·9	*125*	22·7	*439*	30·8	*628*

Means of triplicates of the above treatments expressed as percentages of the maximum response.

Nil ..	4	*5*	4	*4*	4	*4*	4	*4*
Nitrogen ..	24	*24*	22	*23*	77	*81*	100	*100*
Nitrogen plus Minerals	23	*22*	23	*27*	62	*62*	99	*97*

† Inositol, calcium pantothenate, nicotinic acid, pyridoxin, *p*.aminobenzoic acid and biotin.

In group C, the large differences required for significance are due to the considerable variation between triplicates in these treatments where a large response was obtained.

Results of the growth factor experiments on centrifuged ciders of the varieties Kingston Black, Yarlington Mill, Tardive Forestier and Bramley's Seedling are tabulated only as percentages of the treatments giving maximum response, since this is the most concise method of presentation. The tables also show the differences required for significance between means.

The duration of the growth factor experiment on Kingston Black cider was much longer than for those on the other varieties. This was probably due to the very long period of storage of the centrifuged cider before commencing the experiment and to the consequent exhaustion of the yeasts. In spite of this, microscopic examination of the organisms present in different nutrient addition treatments at the end of the experiment showed that the fermentation was due mainly to yeasts, although a few bacteria (lactobaccili) were present. A similar effect was found in a second experiment (not reported here) on Stoke Red cider from Cask 2 after eight months storage.

TABLE III.

DIFFERENCES REQUIRED FOR SIGNIFICANCE BETWEEN MEANS OF TRIPLICATES EXPRESSED AS PERCENTAGES OF THE MAXIMUM RESPONSE (STOKE RED, CASK 2).

Group	Treatments	Significant Difference			
		Fermentation		*Yeast Growth*	
		$P=0{\cdot}05$	$P=0{\cdot}01$	$P=0{\cdot}05$	$P=0{\cdot}01$
A	Omitting Nitrogen	2·9	4.2	*0·6*	*0·9*
B	Containing Nitrogen but omitting Aneurin	2·5	3·6	*5·4*	*7·9*
C	Containing both Nitrogen and Aneurin	18·4	26·8	*33·1*	*48·2*

Discussion.

The graphs showing the utilisation of soluble nitrogen during fermentation for the varieties Stoke Red and Kingston Black are in marked contrast to those for Tardive Forestier and Bramley's Seedling and to those for Bramley's Seedling reported in previous years (1, 2). Both Stoke Red and Kingston Black juices were extremely low in nitrogen content and fermented very slowly. In the Stoke Red juice the greater part of the yeast growth took place during the first ten days and was accompanied by a loss of only 2½ degrees of specific gravity ; in Kingston Black yeast growth was more gradual. The nitrogen contents of the Tardive Forestier and Bramley's Seedling juices were much higher and the yeast growth was accordingly greater. It is unfortunate that for these varieties, no samples were taken during the period of most active yeast growth so that the exact course of the utilisation of soluble nitrogen is uncertain. In spite of this it is apparent that yeast growth was complete at an early stage in the fermentation and for a loss of only six degrees of specific gravity in both juices. The main difference between Tardive Forestier and Bramley's Seedling was the lag-period of eight days in yeast growth in the latter variety ; this could be accounted for by the low temperatures prevailing at that time. The Yarlington Mill juice had a slightly higher nitrogen content than those of Stoke Red and Kingston Black and was intermediate in its fermentation characteristics between these and the higher nitrogen varieties Tardive Forestier and Bramley's Seedling.

In the Stoke Red ciders centrifuged at different specific gravities, yeast growth had gone to completion at time of centrifuging in Casks 4, 5 and 6 and was only a little short of complete in Casks 2 and 3 (soluble nitrogen 1·17 and 1·00 mg.N/100 ml. respectively). All these ciders maintained their specific gravity well throughout the summer, although that in Cask 5 was slightly anomalous.

Yeast growth was also complete at time of centrifuging of the four varieties other than Stoke Red and all these ciders remained fairly stable for six to eight weeks. Subsequently, the Yarlington Mill, Tardive Forestier and Bramley's Seedling ciders all fermented to dryness during the summer but Kingston Black maintained its specific gravity well and was very similar to Stoke Red, Cask 4, which had been centrifuged at approximately the same specific gravity. No simple explanation can be advanced to account for the differences in stability between the ciders of different varieties. It may be that small differences, both qualitative and quantitative, in nitrogen content, growth factors or yeasts present in the centrifuged ciders (and which could not be differentiated by the present analytical techniques) might have a cumulative effect on prolonged storage of the cider. It is suggestive that in

TABLE IV.

KINGSTON BLACK.

Cider centrifuged on 22/1/52, at specific gravity 1,032·0.
Growth Factor Experiment :—
Date commenced 13/5/52 ; duration 23 days.
Initial specific gravity 1,026·1.
Initial yeast content 8 mg./100 ml.
Maximum response, fermentation 13·3 degrees S.G.
Maximum response, yeast growth 458 mg./100 ml.
Results below are means of triplicate determinations and are expressed as percentages of the maximum.

Added Nitrogen or Minerals	Added Growth Factors							
	Nil		Six growth factors		Aneurin		Aneurin plus six growth factors	
	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*
Nil ..	6	*2*	9	*2*	6	*2*	9	*2*
Nitrogen ..	35	*20*	44	*24*	82	*83*	99	*99*
Nitrogen plus Minerals	36	*23*	44	*22*	85	*93*	100	*100*

Differences required for significance between means.

Group	Treatments	Significant Difference Fermentation		*Yeast Growth*	
		P=0·05	P=0·01	P=0·05	P=0·01
A	Omitting Nitrogen	1·0	1·5	*0·4*	*0·5*
B	Containing Nitrogen but omitting Aneurin	4·8	7·0	*4·3*	*6·2*
C	Containing both Nitrogen and Aneurin	9·8	14·3	*7·3*	*10·6*

the growth factor experiments the ciders which were most stable during storage (Stoke Red and Kingston Black) appeared to have a more acute deficiency of nitrogen than of aneurin. In these two ciders the response obtained by the addition of nitrogen in the absence of aneurin was greater than when aneurin was added in the absence of nitrogen, i.e. the absence of assimilable nitrogen was an absolute bar to yeast growth even when aneurin was added. In contrast, in the Tardive Forestier and Bramley's Seedling ciders there was sufficient assimilable nitrogen to allow a little yeast growth when only aneurin was added.

In all the growth factor experiments, with the exception of the varieties Tardive Forestier and Bramley's Seedling, the results given by the different nutrient addition treatments fall clearly into the three groups previously

TABLE V.

YARLINGTON MILL.

Cider centrifuged on 20/12/51, at specific gravity 1,024·6.
Growth Factor Experiment :—
Date commenced 18/1/52, duration 6 days.
Initial specific gravity 1,022·7.
Initial yeast content 13 mg./100 ml.
Maximum response, fermentation 16·2 degrees S.G.
Maximum response, yeast growth 362 mg./100 ml.
Results below are means of triplicate determinations and are expressed as percentages of the maximum.

Added Nitrogen or Minerals	Added Growth Factors							
	Nil		Six growth factors		Aneurin		Aneurin plus six growth factors	
	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*
Nil ..	7	*4*	8	*5*	10	*5*	9	*5*
Nitrogen ..	20	*11*	19	*15*	97	*78*	97	*85*
Nitrogen plus Minerals ..	16	*15*	16	*15*	94	*84*	100	*100*

Differences required for significance between means.

Group	Treatments	Significant Difference Fermentation		*Yeast Growth*	
		P=0·05	*P*=0·01	*P*=0·05	*P*=0·01
A	Omitting Nitrogen	1·2	1·7	*1·2*	*1·7*
B	Containing Nitrogen but omitting Aneurin	1·8	2·6	*5·2*	*7·5*
C	Containing both Nitrogen and Aneurin	12·9	18·8	*23·4*	*34·1*

described, namely : A—treatments omitting nitrogen ; B—treatments containing nitrogen but omitting aneurin ; C—treatments containing both nitrogen and aneurin. With the Tardive Forestier and Bramley's Seedling ciders, in group A the addition of aneurin in the absence of nitrogen gave responses of the same order as those in group B.

With all ciders the main response in both yeast growth and fermentation, was obtained by the addition of both nitrogen and aneurin and this was much greater than that obtained when either was added singly ; a similar result was found with a Bramley's Seedling cider in the previous year. It seems justifiable therefore to conclude that the stability of a naturally sweet centrifuged cider may generally be attributed to a combined deficiency of both nitrogen and aneurin.

TABLE VI.

TARDIVE FORESTIER.

Cider centrifuged on 7/1/52, at specific gravity 1,021·0.
Growth Factor Experiment :—
Date commenced 14/2/52 ; duration 7 days.
Initial specific gravity 1,017·5.
Initial yeast content 7 mg./100 ml.
Maximum response, fermentation 14·1 degrees S.G.
Maximum response, yeast growth 232 mg./100 ml.
Results below are means of triplicate determinations and are expressed as percentages of the maximum.

Added Nitrogen or Minerals	Added Growth Factors							
	Nil		Six growth factors		Aneurin		Aneurin plus six growth factors	
	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*
Nil ..	8	*6*	8	*9*	13	*11*	19	*16*
Nitrogen ..	15	*15*	19	*17*	100	*93*	100	*100*
Nitrogen plus Minerals	17	*14*	18	*16*	97	*95*	98	*97*

Differences required for significance between means.

Group	Treatments	Significant Difference Fermentation		*Yeast Growth*	
		P=0·05	P=0·01	P=0·05	P=0·01
A	Omitting Nitrogen	2·3	3·4	*2·7*	*3·9*
B	Containing Nitrogen but omitting Aneurin	2·6	3·8	*4·1*	*6·0*
C	Containing both Nitrogen and Aneurin	6·3	9·2	*23·0*	*33·5*

Supplementary effects due to growth factors other than aneurin were observed in all ciders except Yarlington Mill. In the Kingston Black cider, the addition of these growth factors increased the fermentation in all groups (A, B and C) and yeast growth in group C. Similar effects were also shown in Stoke Red, group C, in Tardive Forestier, group A, and to a marked extent in Bramley's Seedling, group B. Although these differences were statistically significant they were usually small, so that deficiency of growth factors other than aneurin was only of minor importance in determining the stability of these centrifuged ciders.

Effects due to the addition of minerals were, in general, not significant with the exception of group B of Yarlington Mill where fermentation was depressed. The main purpose of adding minerals was to compare the addition of only nitrogen and aneurin with the relatively complete nutrient addition

TABLE VII.

BRAMLEY'S SEEDLING.

Cider centrifuged on 3/1/52, at specific gravity 1,024·8.
Growth Factor Experiment :—
Date commenced 3/4/52 ; duration 7 days.
Initial specific gravity 1,017·7.
Initial yeast content 10 mg./100 ml.
Maximum response, fermentation 6·5 degrees S.G.
Maximum response, yeast growth 235 mg./100 ml.
Results below are means of triplicate determinations and are expressed as percentages of the maximum.

Added Nitrogen or Minerals	Added Growth Factors							
	Nil		Six growth factors		Aneurin		Aneurin plus six growth factors	
	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*	Fermentation	*Yeast Growth*
Nil ..	14	*9*	16	*11*	22	*14*	21	*14*
Nitrogen ..	17	*17*	26	*25*	100	*100*	98	*89*
Nitrogen plus Minerals	17	*18*	26	*25*	86	*85*	87	*84*

Differences required for significance between means.

Group	Treatments	Significant Difference Fermentation		*Yeast Growth*	
		$P=0·05$	$P=0·01$	$P=0·05$	$P=0·01$
A	Omitting Nitrogen	3·6	5·2	*2·0*	*2·9*
B	Containing Nitrogen but omitting Aneurin	2·3	3·4	*1·1*	*1·7*
C	Containing both Nitrogen and Aneurin	25·2	36·6	*12·6*	*18·4*

of nitrogen, minerals and all seven growth factors. It is remarkable that in all ciders this comparison has shown only small differences and that the stability of the centrifuged ciders should be so unequivocably due to deficiency of nitrogen and aneurin.

Schultz *et al.* (5) have shown that aneurin stimulates the rate of fermentation of bakers' yeast and have used this effect as the basis of a method of aneurin assay. This mechanism however, did not operate in the present ciders since in all the growth factor experiments the fermentation response in each of the treatments was, in general, proportional to the amount of yeast growth which occurred. The addition of aneurin therefore determined the response in fermentation entirely by its effect on yeast growth.

These growth factor experiments were designed to investigate the nutritional deficiencies preventing further growth of the yeasts present in the centrifuged ciders, as far as possible under conditions similar to those in the cask. It was therefore necessary to work with the mixed microflora (yeasts and bacteria) present in the cider and to conduct the tests anaerobically, since under aerobic conditions film forming yeasts and acetic bacteria would certainly have flourished. Little is known of the aneurin requirements of yeasts under anaerobic conditions and it is difficult, therefore, to interpret these experiments in terms of yeast metabolism.

The importance of aneurin in alcoholic fermentation is well known, it forms an essential part of the carboxylase enzyme which decarboxylates pyruvic acid into carbon dioxide and acetaldehyde. Many yeasts are able to synthesise aneurin for growth under aerobic conditions but others require aneurin to be supplied in the growth medium. Atkin *et al.* (6) have studied the growth factor requirements of different strains of brewers' yeasts for purposes of classification. They found that out of 58 yeasts examined, only five—all top-fermenting yeasts required aneurin (or pyridoxin[+]) biotin, pantothenic acid and inositol for growth under aerobic conditions ; the remainder—all bottom yeasts—did not require aneurin or pyridoxin. The yeasts present in the centrifuged ciders were not examined in this way for their growth factor requirements, but whatever their individual requirements under aerobic conditions, it is certain that under the anaerobic conditions of the present growth factor experiments there was collectively a strong requirement for aneurin and for no other growth factor.

In current work it is planned to examine the aneurin requirements of yeasts isolated in pure culture from centrifuged ciders, under both aerobic and anaerobic conditions. In this way, the function of aneurin in determining the stability of ciders will be more clearly seen since the complications arising from the necessity of working, in the first instance, with the mixed microflora present in the ciders will be eliminated. In addition the aneurin requirement of the different yeasts under aerobic conditions can be related to the work of other investigators.

Summary.

(1) The course of yeast growth has been examined in apple juices of five different varieties of low to medium nitrogen content by following the utilisation of nitrogen during fermentation.

[+] Aneurin and pyridoxin were mutually replaceable, a phenomenon which has also been reported for bakers' yeast (7).

(2) In the resulting ciders, yeast growth was substantially complete when they were centrifuged and all remained fairly stable for at least six to eight weeks during storage.
(3) Further yeast growth and fermentation in these centrifuged ciders was limited by a combined deficiency of both nitrogen and aneurin.
(4) It is suggested that the stability of naturally sweet centrifuged ciders in general, can be accounted for by this mechanism.

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THE USE OF SELECTED YEASTS IN CIDER-MAKING : IIA. THE EFFECTS OF FERMENTATION TREATMENTS ON THE YEAST FLORA.

By F. W. BEECH.

Introduction.

In a previous paper an account was given of two seasons' work with selected yeasts in cider-making. Because of the exploratory nature of these experiments the tests for the presence of the selected yeasts in the supernatant ciders at S.G. 1,025 were based on morphological similarities between the selected yeasts and yeasts taken at random after plating out the sample. With few exceptions, each of the selected yeasts achieved dominance during this primary fermentation. In view of the limitations of these methods it was felt that the identity of the yeasts should be established by more detailed tests and also that the ciders should be sampled at a later stage of the fermentation to see whether the selected yeasts still persisted.

Accordingly in this later work, both the supernatant ciders and their yeast lees were sampled at two stages of the fermentation. These samples were plated out quantitatively and after incubation each yeast showing differences in colonial form or microscopical appearance was isolated in pure culture. An assessment was then made of the proportion formed by each of these yeasts of the total number of yeasts present in the sample. Then, using biochemical tests, an attempt was made to prove the identity between the selected yeasts originally added and those isolated in pure culture from the resulting ciders.

During the earlier experiments it was found that the juice treatments required to suppress the yeasts naturally present also affected the course of fermentation and the flavour of the resulting ciders. Hence the later experiments were so designed that the separate effects of juice and yeast treatments could be evaluated.

Experimental.

General Cider-making Procedure.

A large number of cider apple varieties were mixed in such proportions that the final cider would be balanced with respect to acid and tannin.

The fruit was washed, milled, pressed and the first pressings bulked in a large, sterilised Ebon vat. After thorough mixing the juice was divided into five portions and treated as follows :—

(i) Untreated
(ii) 150 p.p.m. sulphur dioxide added
(iii) Centrifuged
(iv) Flash pasteurised at 87° C. (189° F.)
(v) Depectinised, pulp- and sterile-filtered.

Each batch of juice was further divided between three, freshly-steamed, 60-gallon barrels. The first barrel in each group of three was left uninoculated, except in treatments (iv) and (v) where the natural flora had been destroyed or removed: To the first barrel in (iv) and (v), 1 gallon of fresh juice was added to ensure fermentation by the natural flora as in the controls of (i), (ii) and (iii). A pure culture of a cider yeast (TB6) was added to the second barrel of each group, whilst a wine yeast (Champagne) was added to the third. For treatment (ii) separate cultures of the selected yeasts were used, previously acclimatised to 250 p.p.m. sulphur dioxide.

The juices were then allowed to ferment, precautions being taken to prevent cross-infection between barrels when specific gravities were being estimated. At S.G. 1,007, the ciders were centrifuged, clarified (K5 filter sheets), saturated with carbon dioxide, sterile-filtered and bottled aseptically in sterile bottles. Samples of the same ciders were bottled similarly after sweetening to S.G. 1,025 with sugar syrup.

Microbiological Methods.

Production of the Inocula.

Each yeast was increased in successive steps from a loopful off the stock slope to 1,500 ml. The medium used consisted of equal parts of apple juice and tap water fortified with 1% Difco yeast extract, 1% peptone, 0·1% di-ammonium hydrogen phosphate and 0·02% magnesium sulphate, sterilised by autoclaving. For the next stages of inoculum production, the medium was diluted apple juice, 0·05% Difco yeast extract and 0·2% di-ammonium hydrogen phosphate. This mixture was boiled for 15 mins. in a closed, steam-jacketed pan and filled hot into warm, sterilised, 1- and 3-gallon jars. When cool, four of these 1-gallon jars were inoculated using the 1,500 ml. starters and the cultures from the 1-gallon jars were used to inoculate the 3-gallon jars. At all stages the cultures were incubated at 25° C. until active fermentation set in, before transferring to the next stage.

For the SO_2 series the two yeasts were first acclimatised on a test-tube scale to increasing quantities of sulphur dioxide until they grew freely at 250 p.p.m. After acclimatisation, the sterilised medium always contained this quantity of SO_2. The final inocula in the SO_2 series were grown in a 3-gallon jar for each yeast as before.

In the earlier work no attempt was made to standardise the amount of yeast added to the barrels and the culture medium was added with each yeast. It was felt that this latter procedure might influence the flavour of the final ciders so in these experiments, the inocula were centrifuged and the deposits suspended in batches of the same bulk juice used for the main experiment (previously sterile-filtered). Thus for each yeast there were two inocula containing SO_2- and non-SO_2-trained yeasts respectively. The weights of yeast present in the suspensions were calculated from their turbidity (measured photoelectrically by Mr. L. F. Burroughs) and expressed in terms of grams moist Baker's yeast per 100 ml. of culture. The amounts added to the casks, together with the total (haemocytometer) and viable counts are given in Table I.

TABLE I.

QUANTITY OF YEAST PRESENT IN EACH INOCULUM.

	TB6 (no-SO_2)	TB6 (SO_2)	Champagne (no-SO_2)	Champagne (SO_2)
Weight of yeast added to the relevant barrel. (Expressed as g. moist baker's yeast)	75 g.	50 g.	75 g.	75 g.
Yeast concentration in the inocula, as estimated turbidimetrically. (g. moist baker's yeast per 100 ml.)	19·2	10·0	16·7	15·0
Total (Haemocytometer) count per 1 ml.	2580×10^6	1040×10^6	1420×10^6	1140×10^6
Viable yeast per 1 ml.	$6{\cdot}55 \times 10^6$	44×10^6	905×10^6	$116{\cdot}5 \times 10^6$

TB6 did not grow well in the presence of sulphur dioxide, so that a lower concentration of yeast had to be added to this barrel. Calculation of the volume of each yeast cell from the turbidity (line 2, Table I) and the total count shows that TB6 is a smaller yeast than Champagne—as was observed microscopically, but, more important, that the cell size of yeasts grown in the presence of SO_2 was greater than that of the same yeasts in the absence of SO_2. It is intended to carry out further experiments to see whether this increase in cell volume is also associated with any change in fermentative powers.

Although turbidimetric methods afford a rapid means of standardising yeast inocula, they are not entirely satisfactory since : (*a*) two suspensions of yeasts of different size, having the same turbidity, will contain different (total) numbers of yeast cells per 1 ml. and (*b*) there is no means of ensuring at the same time that all the yeasts present are viable. In these experiments only a fraction of the yeasts present in the TB6 cultures and 10% of the Champagne SO_2 cultures were alive, whereas 65% of the Champagne no-SO_2 culture were viable. The effect of this is shown in Table V, which gives the number of viable yeasts per 1 ml. present in each cask after inoculation.

Isolation of the Yeasts in Pure Culture.

Samples were removed aseptically from the five bulks of uninoculated treated juice and from the standardised yeast inocula and plated out after serial dilution on the following medium : Depectinised Bramley's Seedling juice, 1% Difco yeast extract, 0·0001% aneurin (2, 3), adjusted to pH 4·8, 4% agar added, and sterilised by autoclaving at 7 lbs. for 15 minutes. Samples of the supernatant ciders and their yeast lees were also taken aseptically at S.G. 1,025 and 1,007 and plated out. All plates were incubated at 25° C., seven days anaerobically to allow development of anaerobic bacteria (3) and then a further three days aerobically to increase the size of the yeast colonies.

A colony of each yeast showing differences in colonial form was removed from all plates and inoculated into the above medium, without agar. The numbers of each of these yeasts present were counted, together with the total number of yeasts, bacteria and moulds present on the appropriate plate. The liquid cultures were incubated aerobically at 25° C. and purified by streaking twice on to Wickerham's agar medium (4) which has a pH of 6, allowing the development of any bacteria actually present but suppressed at pH 4·8 in the isolation medium. Liquid isolates were taken after streaking and the final cultures were stored on slopes of Wickerham's medium in 1 oz. screw-capped, McCartney bottles and stored at 2° C.

The bottled ciders were only examined for proof of sterility.

Methods for Assessing Dominance.

All the yeasts isolated in pure culture were grown on the apple juice-yeast extract-agar medium and their colonial form and cell shape examined. This made it evident that the fresh juice contained yeasts showing some morphological similarities to the selected yeasts, so that biochemical tests were essential for differentiation purposes.

Thus the yeasts isolated from the ciders at S.G. 1,007 were submitted to the following tests and their reactions compared with those of yeasts added originally in the inocula.

(a) *Alcohol Tolerance.* Tests were made on their ability to utilise 0·5% glucose as a carbon source in a synthetic liquid medium (4) in the presence of 4·8, 6·4 and 8% v/v alcohol respectively. The tests were made in 1 oz. sealed McCartney bottles incubated 14 days at 25° C. Yeast growth was estimated visually.

(b) *Melibiose Fermentation.* A solution of melibiose and yeast extract was prepared from a 4% solution of raffinose and baker's yeast (5). The purity of the melibiose was checked chromatographically before use by Dr. S. C. Agarwala. The ability to ferment melibiose was estimated from the amount of gas formed in inverted Durham tubes at 25° C. The tests were continued for 14 days to allow for lag phase.

(c) *Raffinose Fermentation.* Following Wickerham's method (6), a 4% solution of raffinose containing 0·45% Difco yeast extract and 0·75% peptone was dispensed in ¼ oz. McCartney bottles containing a small, inverted Durham tube. After inoculation and incubation at 25° C. for 28 days the bottles were reinoculated with an active culture of *Saccharomyces carlsbergensis*, to determine whether the original yeast had fermented ⅓ or all of the original raffinose. Wickerham states that some yeasts ferment melibiose more readily when it is presented in the form of raffinose.

(d) *Inhibition by Actidione.* 2, 4, 8 and 25 p.p.m. Actidione plus 2% agar was incorporated in Green and Gray's medium (7) and the yeasts inoculated in duplicate as for a grid plate test. The plates were incubated aerobically at 25° C. for 4 days and the amount of growth produced estimated visually.

(e) *Vitamin Deficiency Test* (4). The growth ability of the yeasts in the absence of vitamins within 7 days was tested in a liquid synthetic medium incubated at 25° C. To differentiate between those yeasts able to grow in the absence of vitamins and those growing by virtue of any vitamins carried over with the inoculum, two drops from each tube showing more than weak growth were added to a second tube and the test repeated.

(f) *Bios Typing Test.* This test was originally developed by R. C. Codner, a former worker at the Research Station, as a grid plate test from Schultz and Atkin's method (8). To their medium was added an equal volume of 4% agar, previously washed as recommended by Beijerinck (9, 10), and each plate was inoculated with 26 yeasts in duplicate. One plate had all the growth factors, in the remaining plates six of the B group of vitamins were omitted singly in turn, with a further plate in which aneurin and pyridoxin were omitted simultaneously. The plates were incubated at 25° C. for 3 days and when growth was absent or less than 50% of that on the "Complete" plate then the yeast was assigned the relevant Bios Number as follows :—

Bios Number (B.N.) 1. .. Restricted growth where inositol omitted
2. .. ,, ,, ,, calcium pantothenate omitted
3. .. ,, ,, ,, biotin omitted
4. .. ,, ,, ,, aneurin omitted
5. .. ,, ,, ,, pyridoxin omitted
6. .. ,, ,, ,, aneurin and pyridoxin omitted
7. .. ,, ,, ,, nicotinic acid omitted

The author found that the test could be made more precise by taking the inocula from the tubes in Test (e) and by standardising the inocula turbidimetrically.

Results.

Although yeasts were isolated from the fermenting juices both at S.G. 1,025 and 1,007 it was considered that only those yeasts that remained viable throughout the fermentation, i.e. until S.G. 1,007, should be compared with the selected yeasts originally added. The account given below specifies the yeasts isolated that were considered to be identical with the added yeasts. The fermentation characteristics, analytical results and the aroma and flavour of the ciders are then examined in the light of the microbiological data to see how far any of these differences can be attributed to the selected yeasts. The effects of juice treatment on the flora and on the ciders are also examined.

Microbiological Observations.

Juice Treatments.

Plate counts were made on the bulks of juices immediately after treatment and before inoculation and the results are given in Table II.

Addition of 150 p.p.m. SO_2 effectively reduced the natural flora since the total numbers of viable yeasts and bacteria were reduced to 1·25% of their initial value. Relatively more bacteria were destroyed than yeasts. On the other hand centrifugation was found unsuitable since yeasts were removed much more readily than bacteria. The increase in the bacterial content of the centrifuged juice compared with the control has been observed before with ciders and it is probable that the numbers of bacteria counted in the untreated juice were less than those actually present due to competition for nutrients

TABLE II.

THE NUMBERS OF VIABLE YEASTS AND BACTERIA PRESENT PER 1 ML. OF JUICE AFTER THE PRE-TREATMENTS.

	Juice Treatments					
	Untreated	Sulphited	Centrifuged	Flash Pasteurised	Sterile filtered	
					Direct from filter	In Barrel
Yeasts ..	400,000	6,500	65,000	245	nil	45
Bacteria ..	200,000	1,500	550,000	315	nil	5

with the faster growing yeasts on the agar plates. Flash pasteurisation was very efficient, destroying 99·9% of the original flora—in these experiments the juice was cooled immediately after the heating cycle, i.e. there was no holding period. Sterile filtration was even more efficient, juice taken direct from the filter being free from micro-organisms, but a small amount of contamination was picked up while the sterile-filtered juice was being distributed amongst the barrels.

Very few bacteria were isolated once fermentation commenced, forming less than 1% of the total viable population at S.G. 1,007.

Tests for Assessment of Dominance.

The TB6 and Champagne yeasts had the characteristics given in Table III.

TABLE III.

CHARACTERISTICS OF THE SELECTED YEASTS TB6 AND CHAMPAGNE.

Yeast	Alcohol tolerance			Fermentation of Raffinose		Growth in presence of Actidione			Growth in Vitamin-deficient medium		Bios Type number
	4·8%	6·4%	8·0%	1/3rd	3/3rds	2 p.p.m.	4 p.p.m.	8 p.p.m.	1st Tube	2nd Tube	
TB6 ..	Weak	—	—	+	+	+	+	+	+	+	3
Champagne	+	+	—	—	—	+	Tr	—	—	—	1.2.3.6.

Neither of the two selected yeasts have been classified into species. TB6, originally isolated by Barker (11) from cider 40 years ago, is a small, circular yeast having many of the characteristics of *Saccharomyces florentinus.* It has only a very slight requirement for biotin. Champagne yeast, isolated by Bunker from champagne, is another ascosporogenous yeast and shares with *Saccharomyces steineri*, which it resembles microscopically, the unusual property of fermenting galactose and sucrose but not raffinose. The Champagne yeast is more exacting than TB6 in its vitamin requirements, needing inositol, pantothenic acid, biotin and either aneurin or pyridoxin, the last two being interchangeable. This yeast forms a pink pigment in the absence of biotin but when methionine is present (17).

As the yeast inocula were not centrifuged aseptically they were examined for the presence of contaminants, using the same tests. 94% of the yeasts in TB6 (no-SO_2) and 90% of those in TB6 (SO_2) were true to type; both Champagne inocula were true to type. None of the contaminants in the TB6 cultures were found at S.G. 1,007 and so, presumably, did not affect the course of fermentation.

Of the 60 yeasts isolated in pure culture at S.G. 1,007, only three had the physiological and morphological characteristics shown for the selected yeasts in Table III. The ciders from which they were isolated, together with the percentages they formed of the total number of yeasts in the supernatant and lees samples respectively are given in Table IV.

TABLE IV.

SELECTED YEASTS ISOLATED FROM THE CIDERS AT S.G. 1,007.

(Expressed as a percentage of the total number of viable yeasts present in each sample).

Yeast	Original Juice Treatment	Supernatant	Lees
TB6	Addition of 150 p.p.m. SO_2	33%	50%
Champagne ..	Flash-Pasteurisation	6%	38%
Champagne ..	Sterile-Filtration	10%	39%

Thus, in these experiments, the added yeasts could not be recovered from the ciders at S.G. 1,007 unless the original flora had been greatly reduced, i.e. treatments (ii), (iv) and (v). In no case did the selected yeasts survive in the untreated juice at the concentrations added originally. The distribution of the Champagne yeast was as reported previously (1) i.e. it showed a tendency to flocculate towards the end of the fermentation.

None of the selected yeasts were completely responsible for the fermentations, being overgrown to a greater or less extent by the natural juice flora. The other yeasts isolated from the ciders came from the juice originally and could be distinguished readily from the selected yeasts as follows :—

Cider yeasts having same cell shape as TB6 :—

> Greater tolerance of alcohol, no fermentation of raffinose, no growth in absence of vitamins in the liquid medium, no growth on the solid medium in absence of inositol and biotin.

Cider yeasts having the same cell shape as Champagne :—

> Ferment melibiose completely, no growth in presence of Actidione, growth in absence of vitamins in liquid medium, no growth on the solid medium in absence of inositol.

Lemon-shaped cider yeasts :—

Very low tolerance of alcohol, no fermentation of raffinose, unaffected by Actidione, no growth in absence of vitamins in liquid medium, requires all the six vitamins for growth on the solid medium.

The colonial appearance of the selected yeasts, of the yeasts isolated from the ciders and identical with them and of the three examples of cider yeasts are shown in Plate I. The bios typing tests for the same yeasts are shown in Plate II.

Changes in the Numbers of Viable Yeasts During Fermentation.

Viable (plate) counts on the yeasts in suspension are truly representative for the supernatant liquid in the barrel (3, 12) but viable counts on the lees samples can only be used to determine the proportion formed by each type of yeast in a particular sample, the total volume of lees in the barrel being unknown. Table V shows the changes in the suspended viable yeasts during fermentation.

TABLE V.

CHANGES IN THE NUMBERS OF VIABLE YEASTS PER 1 ML. OF SUPERNATANT CIDER DURING FERMENTATION.

(all values to be multiplied by 10^4).

	Juice Treatment				
	Untreated (i)	Sulphited (ii)	Centrifuged (iii)	Flash-Pasteurised (iv)	Sterile-Filtered (v)
Uninoculated					
Fresh Juice ..	40	0·7	6·5	0·7	0·6
S.G. 1,025 ..	60	3150	49	515	665
S.G. 1,007 ..	No sample	490	0·6	89·5	135
TB 6					
Fresh Juice ..	40·9	8·4	7·4	1·0	0·9
S.G. 1,025 ..	115	1060	160	620	770
S.G. 1,007 ..	45·5	7600	57	116	180
Champagne					
Fresh Juice ..	194	21·6	150·5	162·5	141·8
S.G. 1,025 ..	135	3000	101·5	365	360
S.G. 1,007 ..	4·9	250	No sample	81·5	350

Addition of 150 p.p.m. SO_2 to 60 gallons of juice reduced the numbers of viable yeasts to those present in 1 gallon of untreated juice. Although centrifuging the fresh juice reduced the numbers of yeasts initially present, the changes in the numbers of viable yeasts during fermentation were very similar to those found in the untreated juices. Juice treatments which destroyed or removed most of the natural flora, viz. (ii), (iv) and (v), caused major changes in the numbers of suspended yeasts during fermentation, as shown by the great number at S.G. 1,025 and still considerable numbers at S.G. 1,007 compared with treatments (i) and (iii) at the same gravities.

As shown earlier, addition of the TB6 (no-SO_2) culture hardly raised the number of viable yeasts above those in the corresponding uninoculated controls. There were considerably more yeasts in the TB6-inoculated, sulphited juice than in the SO_2 control. Both the Champagne (SO_2) and (no-SO_2) inocula were also satisfactory.

The addition of the inocula only altered the distribution of yeasts in two of the ciders where selected yeasts were shown by other tests to be actually present, i.e. in TB6 (SO_2) and Champagne-sterile-filtered. Thus the juice treatment had a greater influence on yeast distribution than the selected yeasts themselves.

Fermentation Characteristics.

The rates of fermentation in barrel at the same cider-house temperatures for the fifteen juices are given in Table VI.

TABLE VI.

RATES OF FERMENTATION IN BARREL.

Fresh Juice to S.G. 1,007.

(All results expressed as degrees S.G. lost per 10 days).

Yeast Treatment	Juice Treatment				
	Untreated (i)	Sulphited (ii)	Centrifuged (iii)	Flash-Pasteurised (iv)	Sterile-Filtered (v)
Uninoculated ..	4·8	5·2	4·0	6·6	3·5
TB6	6·4	6·6	5·9	6·0	3·5
Champagne ..	3·9	5·4	2·9	2·9	2·6

In the uninoculated series, addition of SO_2 and flash pasteurisation (plus 1 gallon fresh juice) increased the rate of fermentation, while the other two treatments caused a slight decrease.

From the rates of fermentation it might therefore be assumed that the following fermentations had been dominated by the selected yeasts.

TB6 .. .. .. (i), (ii), (iii).
Champagne .. .. (i), (iii), (iv), (v).

Except in the fermenting juices already shown to contain the selected yeasts, there were no yeasts having the characteristics of TB6 in the ciders from untreated and centrifuged juices originally inoculated with yeast. Similarly in the second group, Champagne yeast appeared absent in cider from the inoculated, untreated juice. 5% of the yeasts in the supernatant cider from centrifuged juice, originally inoculated with Champagne yeast had the same morphological appearance as Champagne yeast but this was not checked microbiologically. Unfortunately the lees sample was lost before plating out, and as the Champagne yeast flocculates, this was the more important sample of the two. Hence it was not possible, from biochemical tests, to say whether the Champagne yeast was present but other evidence suggests that it was probably present and influenced the course of the fermentation.

Chemical Observations.

As might be expected from the small percentages of selected yeasts found in the three ciders, very little chemical evidence of dominance was obtained. However, chromatographic examination of the organic acids at different stages showed that the cider, TB6-SO_2, at S.G. 1,007 had a lower succinic acid content than the other two ciders of that set and further, that the cider, Champagne-flash-pasteurised, at the same gravity had a greater amount than the other two ciders.

The juice treatments had a far greater effect in determining the organic acid changes in the juices and ciders during fermentation than the selected yeasts. These results are discussed in detail in the accompanying paper (13).

Vintage Quality of the Ciders.

The ciders at S.G. 1,007 were saturated with carbon dioxide and sterile-filtered so that there were no changes in flavour during storage such as were experienced in previous years following natural conditioning. The vintage quality of the ciders was assessed by five members of the Cider Section (14, 15) and the sampling was arranged so that the separate effects of juice and yeast treatments could be investigated separately.

When the five dry ciders made from unyeasted juices were compared, it was found that cider made after addition of sulphur dioxide to the juice had the best vintage quality. That from untreated juice was next in quality being a pleasant average cider.

Flash pasteurisation caused no "pasteurised" flavour but gave an unusual cider which had a physical sensation of "softness" in the mouth, probably due to the pectin present. The three ciders made from flash-pasteurised juice were found to contain pectin—in all the ciders of the other series the pectin had been removed by the pectin esterase of the juice.

Sterile-filtered juice gave a thin and rather characterless cider while that from centrifuged juice had a poor aroma and flavour.

The addition of selected yeasts caused only slight changes in the aromas and flavours of the dry ciders. Of the TB6 series, the cider made from sulphited juice was preferred. In the Champagne series, the cider from centrifuged juice was considered the best.

The sweetened ciders showed the same effects of juice treatment although to a slightly less extent and any differences due to the selected yeasts were very small and difficult to detect consistently.

Discussion.

In these experiments five equal portions of a bulk of juice were subjected to different juice treatments, and each portion was further divided amongst three barrels to which different selected yeasts were added. At S.G. 1,007 an examination was made of the yeasts present together with the fermentation characteristics, chemical composition and vintage quality of the ciders produced. The evidence for the presence of the selected yeasts in seven of the ten ciders from inoculated juices can be summarised as follows :—

Yeast	Juice Treatment	Morphology	Biochemical Tests	Distribution of yeasts in supernatant ciders	Rate of Fermentation in Barrel	Acids Produced	Flavour of Dry Ciders
TB6	Sulphited	✓	✓	✓	✓	✓	✓
TB6	Untreated	✓			✓		
TB6	Centrifuged	✓			✓		
Champagne	Untreated	✓			✓		
Champagne	Centrifuged	✓	No sample		✓		✓
Champagne	Flash-Past'ed	✓	✓		✓	✓	
Champagne	Sterile-Filt'ed	✓	✓	✓	✓		

The results indicate that TB6 played an effective part in the fermentation of sulphited juice and still formed approximately 50% of the viable yeasts at S.G. 1.007. The evidence for TB6 in the ciders from untreated and centrifuged juices was only slight, and could not be confirmed by tests based on the biochemistry of the yeasts present.

In the Champagne-yeasted series, there is good evidence for the presence of this yeast in the ciders from flash-pasteurised and sterile-filtered juices. In both ciders this yeast was found almost entirely in the lees. In further work on this topic, the fermenting juices are being stirred thoroughly before sampling, making possible an accurate count of the total viable yeasts. There was some evidence that the Champagne yeast affected the fermentation of the centrifuged juice but confirmation from biochemical tests was not possible. Evidence for the presence of this yeast in the cider from untreated juice was only slight.

Proof of the presence of a selected yeast in a cider should thus be based on several tests, the chief of which are its biochemical reactions, since the juices and ciders contain yeasts that are very similar morphologically to the selected yeasts. Wiles, in an examination of yeasts occurring in a brewery (16), has found that such tests are vital for the correct identification of closely related yeasts.

The failure of TB6 (no-SO_2) to dominate the natural flora is understandable because of the small number of viable yeasts added in the inoculum. The Champagne yeasts survived in a number of the fermentations but even then did not form more than 40% of the total yeast flora. Possibly this was due to their requirement for several growth factors, whereas the morphologically similar yeasts from the juice only required inositol. The experiments show clearly that even though juice treatments such as addition of SO_2, flash-pasteurisation and sterile-filtration remove the natural yeasts almost completely, under large-scale cider-making conditions the few remaining yeasts can still develop and eventually form a major part of the cider yeast flora. A selected yeast must therefore have less exacting nutritional requirements than the natural yeasts as well as potential effect on flavour and fermentation.

While the yeast treatments caused only minor changes, the juice treatments caused major changes in flavour and fermentation characteristics. These, briefly, were as follows :—

(a) 150 p.p.m. SO_2 suppressed the malo-lactic bacteria completely whereas these bacteria destroyed the malic acid in the other ciders.

(b) SO_2 suppressed the yeast flora adequately for practical purposes ; centrifugation was unsatisfactory since it did not reduce the bacterial flora and, without an added yeast, gave a poor quality cider ; flash-pasteurisation and sterile-filtration were very satisfactory for natural yeast suppression. Flash pasteurisation of fresh juice should be avoided without a preliminary depectinisation, as the natural pectin enzymes which normally clarify the cider are destroyed, making subsequent filtration more difficult.

(c) Juice treatments caused great changes in the rates of fermentation of the juices.

(d) Finally the flavours of the ciders were greatly affected by juice treatment, the addition of SO_2 to the juice, by its selective action on the natural flora, again giving the best flavour.

Thus no investigation of the use of selected yeasts can be considered complete without an appreciation of the additional effects of the juice treatments used to suppress any part of the natural flora.

Summary.

(1) In further investigations of cider fermentations using selected yeasts an examination has also been made into the effects of five different juice treatments and their efficiency in reducing the natural flora. The conclusions were as follows :—

(a) Addition of 150 p.p.m. SO_2 was an efficient and practicable method of treatment and the ciders subsequently produced were superior in flavour, as found in previous years.

(b) Flash pasteurisation and sterile-filtration were even more effective but less practicable and did not give high quality ciders.

(c) Centrifugation (of juice) for this purpose was neither efficient nor desirable.

(2) Yeasts were isolated in pure culture from the ciders at S.G. 1,007 and on the basis of biochemical tests and other evidence, it was considered that the selected yeasts were present in large numbers in three out of the ten ciders and possibly in a fourth.

(3) The selected yeasts could only be recovered from the ciders when the original flora had been effectively suppressed, viz. by the use of SO_2, flash pasteurisation or sterile-filtration.

(4) The juice treatments caused major changes in the fermentations and in the flavour of the ciders, whereas, in these experiments, the selected yeasts were responsible for small changes.

(5) Information has been obtained which is being applied to a study of normal cider fermentations.

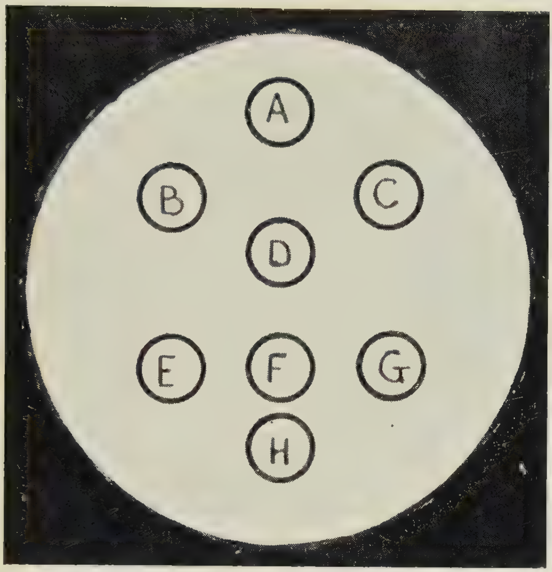


Fig. 1. Code Card showing position of the different yeasts on all the plates.
(A) Lemon-shaped yeast from the uninoculated ciders.
(B) TB6 yeast from the inoculum.
(C) TB6 yeast re-isolated from cider made from sulphited juice.
(D) Yeast resembling TB6 from uninoculated cider.
(E) Champagne yeast from the inoculum.
(F) Champagne yeast re-isolated from cider made from flash pasteurised juice.
(G) Champagne yeast re-isolated from cider made from sterile filtered juice.
(H) Yeast resembling Champagne from uninoculated cider.

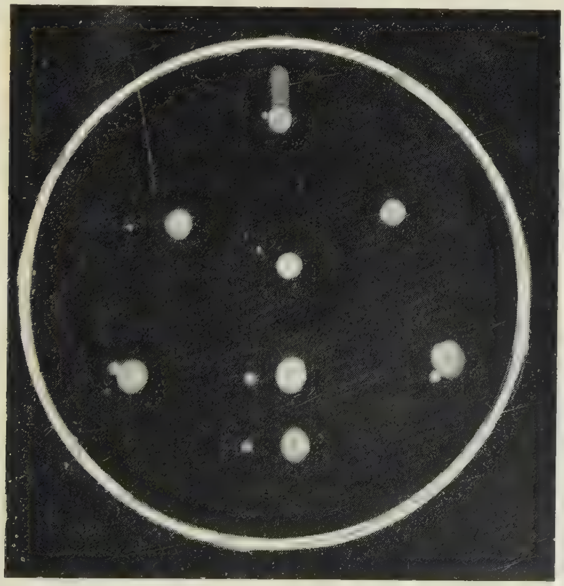
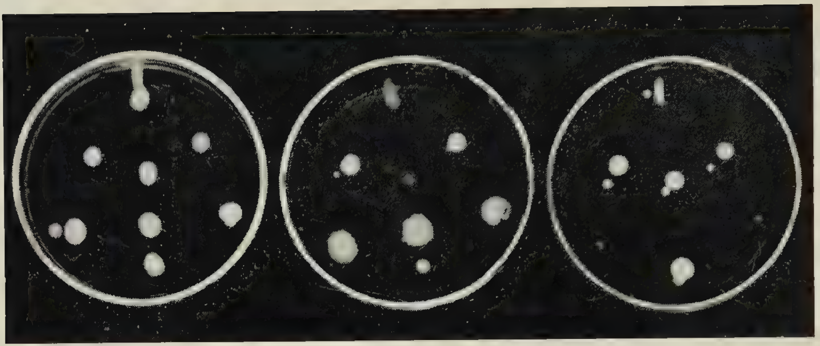


Fig. 2. Appearance of the yeast colonies on the apple juice-yeast extract-agar medium.

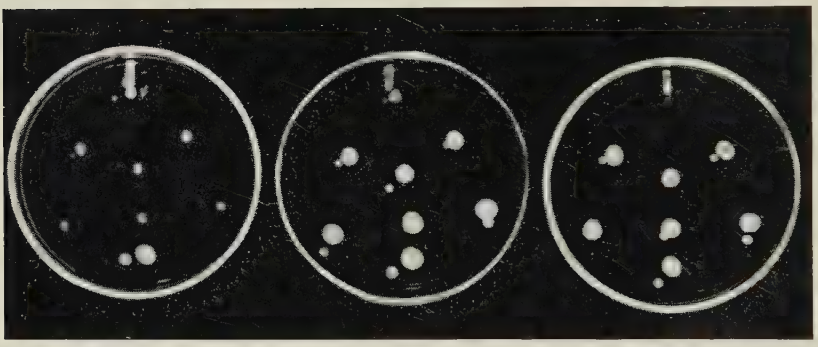
PLATE XI.



Complete

Minus
Inositol

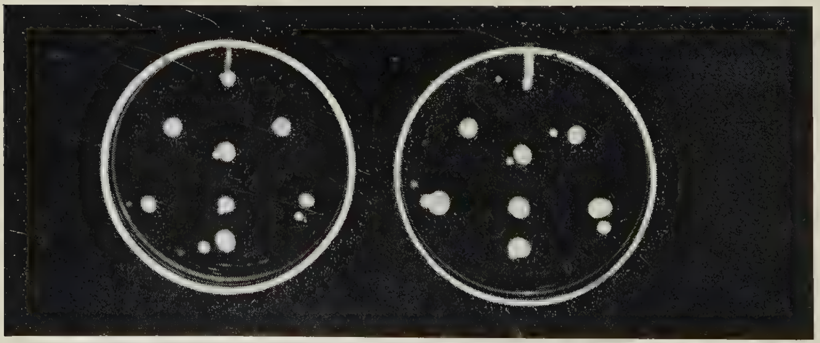
Minus
Pantothenate



Minus
Biotin

Minus
Aneurin

Minus
Pyridoxin



Minus
Aneurin and
Pyridoxin

Minus
Nicotinic
Acid

Fig. 3. Bios Typing Test for yeasts shown on Plate X.

Acknowledgments.

The author wishes to express his thanks to Dr. A. Pollard for his help in planning the experimental work and to Messrs. A. P. V. Co. Ltd., for the loan of a " Paraflow " Heat Exchanger.

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THE USE OF SELECTED YEASTS IN CIDER-MAKING : IIB. THE EFFECTS OF FERMENTATION TREATMENTS ON THE ORGANIC ACID COMPOSITION OF CIDERS.

By J. D. PHILLIPS.

Introduction.

Little systematic investigation has been carried out on the organic acid constitution of English cider apple juices and the changes in acid composition taking place during fermentation. Most of the previous work on organic acids in cider has been concerned with studies of the conversion of malic acid to lactic acid by malo-lactic bacteria ; this aspect is reviewed more fully elsewhere in this Report (1). Recent work by Jacquin and Tavernier (2, 3) has given much valuable information on the organic acid changes taking place during the fermentation of French cider apple juices. The classical methods of analysis used by these workers, although giving accurate quantitative results, suffer from the disadvantage of requiring somewhat detailed and prolonged manipulation for each of the acids to be analysed and also fail to give a complete estimate of the principal organic acids present in apple juice and cider. Quinic acid, recently isolated by Hulme (4) from Worcester Pearmain apples, and shown in this investigation to be present also in cider apple varieties, is not taken into account by these analytical methods and probably forms the greater part of the unidentified organic acid fraction in the analyses carried out by the French workers.

An account is given here of the non-volatile organic acid constitution of the apple juices and ciders discussed in part (A) of this paper. This work is part of a larger programme on the variation of organic acid composition with apple variety, stage of maturity and other factors which will be fully reported elsewhere together with the experimental procedures used in the qualitative separation and quantitative estimation of the acids.

Experimental.

Paper partition chromatography (descending) was used to separate the organic acids, the method being based on that developed by Consden, Gordon and Martin (5) as modified for organic acids by Lugg and Overell (6). By suitable choice of developing solvents it has been found possible to separate all the principal organic acids present in apple juice and cider.

In order to obtain an extract containing free organic acids and to remove substances such as sugars and tannins which will interfere with the chromatographic development, the juice or cider samples were submitted to ion exchange treatments using Amberlite IR 120, Cation exchange resin and Amberlite IRA 400 (CO_3'') anion exchange resin. It is of the greatest importance, in work of this kind involving solutions containing sugars, that strongly basic anion exchange resins of the Amberlite IRA 400 type should not be used in the OH' form but in a less reactive form such as the CO_3'' form (7) (8).

Quantitative results were obtained by running on the same chromatogram the solution under test plus a series of standards containing 20, 30, 40, 50 and 60 μg. of the pure acid to be determined. After development and spraying, the appropriate spots were cut out, dried and weighed. A graph was constructed relating the weights of standard acid applied to the chromatogram with the weight of spot excised from the chromatogram after development. The weight of the corresponding excised spot in the sample under test was compared with the standard graph and an estimate made of the amount of acid present. Under carefully controlled conditions the method is capable of giving an accuracy of the order of ± 5% for acids present at a concentration of greater than 0·2% and ± 10% in the range 0·05 to 0·2%.

The materials examined were apple juice and cider samples from the experiment on the use of selected yeasts fully detailed in part (A) of this paper. In this experiment a blended apple juice was subjected to four different treatments designed to reduce the natural microflora to a very low level.

The juice treatments were as follows :—

Series I .. .. Untreated Control.
II .. .. SO_2 treated.
III .. .. Centrifuged.
IV .. .. Flash pasteurised.
V .. .. EK filtered.

Juice from each of the four treatments plus an untreated control was then divided into three portions for the three yeast treatments. The following samples for acid analysis were taken for each combination of juice treatment and yeast inoculum :—

(1) Juice immediately after treatment (November 1951).
(2) Ciders at S.G. 1,007 (January to April 1952, depending on juice treatment and yeast added).
(3) Dry cider on the lees (August to September 1952).

Small samples of cider were removed from the main bulks at S.G. 1,007 and allowed to continue fermenting in contact with the lees.

Microbiological results show in fact that the added yeast inoculum failed to maintain a dominant position throughout the fermentation and in only three of the S.G. 1,007 ciders was there any quantity of the added yeast species detected. Hence the results reflect differences due mainly to juice treatments, the fermentation being substantially due to a mixed microflora. Each of the samples was prepared for chromatographic analysis by the methods briefly outlined above. Malic, quinic, lactic, succinic, phosphoric, chlorogenic and galacturonic acids were quantitatively estimated by excision of spots and weighing. Volatile acidity was determined by steam distillation in a Markham apparatus and titration of the distillate with N/60 NaOH. Titratable acidity was determined by the method of Burroughs (9) and total acidity by the same method on a sample freed from cations by treatment on a cation exchange resin (Amberlite IR 120).

Summary of Results.

The accompanying Tables (I, II and III) summarise the results of the acid analyses. Volatile acid is expressed as percentage acetic acid. Titratable and total acids are expressed as percentage malic acid. " Total acid accounted for " is also expressed as percentage malic acid and is obtained by converting each of the separately determined acids to their malic acid equivalent and summing them. It will be seen that the " Total acid accounted for " is in the majority of cases within ± 5% of the total acidity found by titration. In the dry ciders the figures in brackets in the lactic acid column represent the lactic acid theoretically to be expected from the observed disappearance of malic acid.

In the chromatogram (Plate XII) a comparison can readily be made of the acid composition of the juice prior to fermentation with that of the cider at S.G. 1,007 and dry ciders from a juice with, and without, treatment with sulphur dioxide.

Discussion.

The acidity of the juices was due mainly to malic acid with smaller amounts of quinic and phosphoric acids, the latter probably being present in the juice in the form of a potassium salt. Quinic acid, although present at a concentration of 0·15% (approx.), contributes an acidity equivalent only to 0·04% malic acid. Small amounts of chlorogenic, galacturonic and succinic acids were also present. As was to be expected there was significantly higher galacturonic acid in the enzymed juice (Series V).

At S.G. 1,007 all ciders show a rise in the total acidity as a result of fermentation. This increase is due mainly to the production of succinic acid as a result of yeast metabolism and to a small extent to increases in the galacturonic acid and volatile acid content. There was a significantly lower succinic acid production in the Series IIB (Yeast TB6) cider than in the other two ciders of this Series. Series IVC (Champagne yeast) contained significantly more succinic acid than the other two Series IV ciders.

The 15 dry ciders examined show in a very striking way how a malo-lactic fermentation can be prevented by the use of SO_2. In all the ciders of Series I, III, IV and V a malo-lactic fermentation has taken place and has gone almost to completion in every case. In the three ciders of Series II, in which the juice had been treated with 150 p.p.m. of SO_2, no malo-lactic fermentation has taken place. There was in most instances a close agreement between the observed lactic acid produced and that theoretically to be expected from the measured disappearance of malic acid.

The amounts of succinic acid are all approximately the same and the values are little different from those found for S.G. 1,007 ciders. Those ciders which at S.G. 1,007 showed a lower succinic acid value than the control have at dryness attained the same value as the others. In these ciders it is probable either that the juice treatment produced an initial retardation of yeast metabolism or that the yeast with which the juice was inoculated may have been a less active succinic acid producer than the natural yeast flora which became dominant in the later stages of the fermentation.

In Series II there is no change in the galacturonic acid content compared with the values at S.G. 1,007. This is also the case in some of the ciders in Series I, III. IV and V, but other ciders of these series show small decreases in galacturonic acid.

It seems clear that to a greater or less extent galacturonic acid is being metabolised in these ciders, though the identity of the organism responsible has not been established.

Quinic, phosphoric and chlorogenic acids appear to be substantially unchanged throughout the fermentation, although it is probable that in the early stages there was an uptake of phosphate, which was subsequently released on yeast autolysis.

In almost all the ciders there was a marked increase in volatile acidity, as no special precautions were taken to prevent acetification in these experimental samples drawn from the main bulk during the fermentation from S.G. 1,007 to dryness. However, it is interesting to note that under conditions where a very considerable change in the acid components takes place the methods outlined in this paper are capable of following the changes with an error of less than 5%. This is so in Series IIIA in which at S.G. 1,007 the main acid of this cider was malic acid with very little volatile component whereas at dryness all the malic acid had disappeared and the acidity was mainly due to lactic acid and the volatile acid fraction.

Summary.

(1) Malic acid is the dominant organic acid of the apple juice investigated : there are smaller amounts of quinic acid and very small quantities of chlorogenic, galacturonic and succinic acids.
(2) An increase in the succinic acid fraction was the only change observed in the acid composition as a result of yeast fermentation.
(3) Bacterial malo-lactic fermentation was general except where prevented by the use of sulphur dioxide at a level of 150 p.p.m.

TABLE I.

ORGANIC ACID CHANGES DURING CIDER FERMENTATION.

LEGEND.
Series I = Juice untreated (Control).
II = Juice treated with 150 p.p.m. SO_2.
III = Juice centrifuged.
IV = Juice flash pasteurised.
V = Juice EK filtered.

Yeast A = Normal yeast.
,, B TB6 ,,
,, C = Champagne ,,

Titratable acid } Expressed as % malic acid.
Total acids }

Series	Yeast	Juice (Nov. 1951)										
		% Malic acid	% Lactic acid	% Succinic acid	% Quinic acid	% Phosphoric acid	% Chlorogenic acid	% Galacturonic acid	Volatile Acid as % Acetic	Total Acid† accounted for	By Titration	
											Total Acid†	Titratable Acid†
I	A B C	0·44	nil	Trace	0·13	0·02	0·05	Trace	0·003	% 0·54	% 0·59	% 0·38
II	A B C	0·45	nil	Trace	0·10	0·02	0·055	0·03	nil	* 0·575	0·62	0·40
III	A B C	0·43	nil	Trace	0·13	0·02	0·055	Trace	0·003	0·53	0·58	0·38
IV	A B C	0·44	nil	Trace	0·12	0·02	0·055	0·01	0·003	0·54	0·59	0·39
V	A B C	0·45	nil	Trace	0·11	0·02	0·055	0·065	0·004	0·57	0·60	0·39

* Total acid values in Series II include a correction for the SO_2 present.
† Expressed as % malic acid.

TABLE II.

ORGANIC ACID CHANGES DURING CIDER FERMENTATIONS.

Series	Yeast	S.G. 1,007 Cider (Jan.–April 1952)										
		% Malic acid	% Lactic acid	% Succinic acid	% Quinic acid	% Phosphoric acid	% Chlorogenic acid	% Galact-uronic acid	Volatile Acid as % acetic	Total Acid† accounted for	By Titration	
											Total Acid†	Titratable Acid†
										%	%	%
I	A	0·45	nil	0·075	0·16	0·02	0·05	0·075	0·001	0·67	0·71	0·52
	B	0·46	nil	0·07	0·13	0·02	0·05	0·075	0·011	0·67	0·69	0·49
	C	0·43	nil	0·08	0·14	0·02	0·05	0·075	0·014	0·66	0·71	0·54
II	A	0·47	nil	0·075	0·13	0·02	0·05	0·075	0·01	0·71*	0·73	0·53
	B	0·47	nil	0·05	0·15	0·02	0·05	0·075	0·014	0·69*	0·66	0·46
	C	0·47	nil	0·07	0·15	0·02	0·05	0·075	0·01	0·72*	0·74	0·55
III	A	0·45	nil	0·07	0·14	0·02	0·05	0·075	0·015	0·67	0·68	0·48
	B	0·42	nil	0·06	0·09	0·02	0·05	0·075	0·025	0·62	0·64	0·50
	C	0·43	nil	0·07	0·10	0·02	0·05	0·075	0·02	0·64	0·66	0·50
IV	A	0·46	nil	0·05	0·10	0·02	0·05	0·045	0·035	0·65	0·665	0·51
	B	0·46	nil	0·06	0·13	0·02	0·05	0·040	0·025	0·66	0·65	0·52
	C	0·44	nil	0·076	0·15	0·02	0·05	0·05	0·03	0·68	0·66	0·49
V	A	0·46	nil	0·07	0·13	0·02	0·05	0·075	0·01	0·67	0·675	0·50
	B	0·48	nil	0·07	0·15	0·02	0·05	0·075	0·005	0·69	0·71	0·54
	C	0·47	nil	0·08	0·12	0·02	0·05	0·075	0·015	0·69	0·70	0·52

* Total acid values in Series II include a correction for the SO_2 present.

† Expressed as % malic acid.

TABLE III.

ORGANIC ACID CHANGES DURING CIDER FERMENTATIONS.

Series	Yeast	Dry Cider (on the lees) (Aug.–Sept. 1952)										
		% Malic acid	% Lactic acid*	% Succinic acid	% Quinic acid	% Phos-phoric acid	% Chloro-genic acid	% Galact-uronic acid	Volatile Acid as % Acetic	Total Acid† accounted for	By Titration	
											Total Acid†	Titratable Acid†
										%	%	%
I	A	<0·01	0·35 (0·32)	0·07	0·13	0·02	0·06	0·07	0·024	0·48	0·47	0·28
	B	<0·01	0·38 (0·32)	0·08	0·12	0·02	0·06	0·07	0·103	0·57	0·55	0·35
	C	0·02	0·29 (0·31)	0·09	0·09	0·02	0·06	0·045	0·126	0·56	0·58	0·38
II	A	0·45	Trace	0·08	0·12	0·02	0·06	0·08	0·087	0·745+	0·78	0·58
	B	0·40	Trace	0·08	0·13	0·02	0·06	0·075	0·136	0·745+	0·70	0·50
	C	0·49	Trace	0·065	0·14	0·02	0·06	0·085	0·080	0·77 +	0·79	0·59
III	A	0·015	0·29 (0·31)	0·065	0·11	0·02	0·06	0·06	0·324	0·745	0·78	0·58
	B	<0·01	0·30 (0·315)	0·07	0·11	0·02	0·06	0·04	0·062	0·48	0·49	0·29
	C	<0·01	0·30 (0·315)	0·08	0·13	0·02	0·06	0·08	0·072	0·505	0·53	0·32
IV	A	0·02	0·30 (0·31)	0·08	0·12	0·02	0·06	<0·02	0·067	0·48	0·48	0·30
	B	0·02	0·30 (0·31)	0·09	0·11	0·02	0·06	<0·02	0·074	0·50	0·50	0·30
	C	<0·01	0·33 (0·32)	0·07	0·11	0·02	0·06	0·02	0·189	0·61	0·61	0·41
V	A	<0·01	0·325 (0·33)	0·08	0·12	0·02	0·06	0·08	0·019	0·47	0·47	0·27
	B	0·01	0·33 (0·33)	0·08	0·13	0·02	0·06	0·06	0·061	0·52	0·51	0·30
	C	<0·01	0·33 (0·33)	0·10	0·13	0·02	0·06	0·065	0·035	0·47	0·50	0·30

+ Total acid values in Series II include a correction for the SO_2 present.

* Figures in brackets indicate the lactic acid theoretically to be expected from the measured disappearance of malic acid.

† Expressed as % malic acid.

Acknowledgments.

I should like to acknowledge my indebtedness to Dr. A. Pollard for his frequent advice and encouragement during this investigation and to Mr. G. H. Jones, A.R.P.S., for the photographic record of the chromatogram.

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THE LACTIC ACID BACTERIA OF CIDER: I. SOME ORGANISMS RESPONSIBLE FOR THE MALO-LACTIC FERMENTATION.

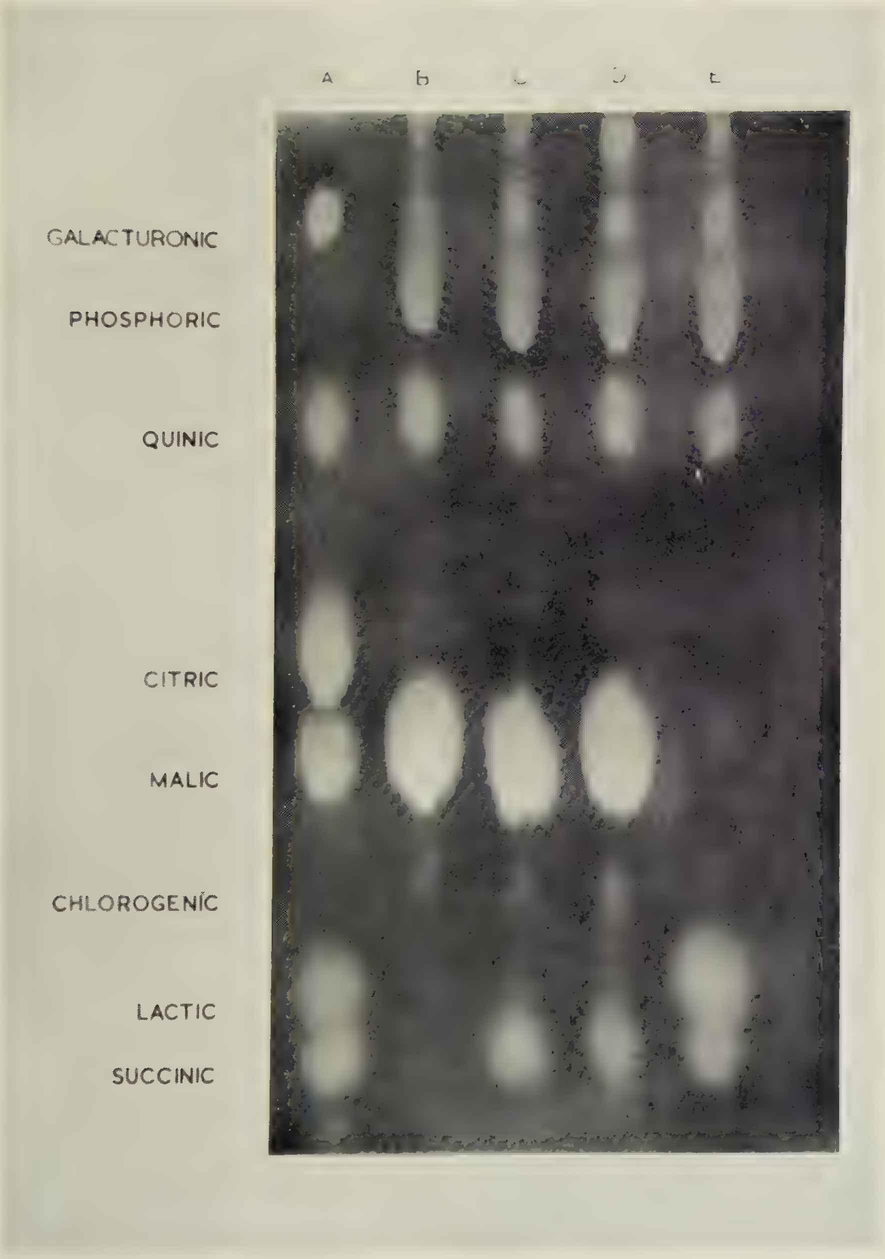
By J. G. CARR.

Introduction.

The reduction of acidity in wines, ciders and perries, brought about by the breakdown of malic acid to lactic acid, has been mainly studied in Switzerland and to a smaller extent in France and America. Although the chemical aspects of this phenomenon have been widely studied, little attention has been paid to the microbiology, with the notable exception of the work at Wädenswil by Müller-Thurgau and Osterwalder (1) who made an extensive study of the subject. They isolated numerous strains of malo-lactic bacteria and classified them under the following names : *Bacterium gracile*, *Bacterium mannitopoeum* (*Lactobacillis buchneri*), *Micrococcus acidovorax* and *Micrococcus variococcus.* Not only did these workers isolate the organisms in pure culture, but they re-inoculated them into wines, ciders and perries and studied their effects upon the composition and quality of these beverages. Of the four species examined, *B. gracile* was found to be the most useful in bringing about a deacidification, with the minimum of harmful effects. *Bacterium mannitopoeum*, by contrast, simultaneously decomposed both sugars and malic acid under certain conditions, producing high levels of mannitol, lactic acid and volatile acid. These changes were associated with undesirable flavours and produced a disorder of the inoculated beverages.

There have been few references to lactic fermentations in English cider-making. The papers of Steuart (2) and Marshall (3) were concerned mainly with the chemical changes rather than micro-organisms, although Marshall, referring to the work of the Swiss school, suggested that *B. gracile* was the organism most frequently responsible for these marked acid changes in English

PLATE XII


A
b
c
D
E
GALACTURONIC
PHOSPHORIC
QUINIC
CITRIC
MALIC
CHLOROGENIC
LACTIC
SUCCINIC

CHROMATOGRAMS OF APPLE JUICE AND CIDER ACID EXTRACTS.

A. Standard mixture containing galacturonic, quinic, citric, malic, lactic and succinic acids.
B. Extract of acids from apple juice (Series I).
C. " " " " cider S.G. 1,007 (Series I).
D. " " " " dry cider (Series II—SO_2 treated).
E. " " " " dry cider (Series V—non-SO_2 treated).

ciders. He commented that, "the malo-lactic fermentation of cider has not received the attention it deserves."

The work to be described forms part of a survey of the lactic acid bacteria encountered in English ciders. The present discussion will be limited to a description of two malo-lactic organisms, their properties and their significance in cider-making.

Methods and Materials for Isolation.

Media for primary isolation. The medium used for this purpose was prepared according to the procedure of Millis (4) and consisted of depectinised apple juice fortified with 1% Difco yeast extract (AJYE). The preparation of the medium was modified by the alteration of the pH from 4·5 to 4·8, and the concentration of agar in the solid medium was raised from 3%–4%. By these slight alterations it was possible to obtain a firmer gel with the solid medium.

Medium for the selection of malate decomposers. To ensure that the organisms selected from the primary isolation plates were capable of splitting malate, they were finally inoculated into the following liquid medium : $\frac{1}{2}$% Difco yeast extract, $\frac{1}{2}$% L-malic acid plus bromo-cresol green indicator, pH adjusted to 4·0. Growth of the organisms in this medium was indicated by slight turbidity and a change of indicator from yellowish green to a well defined green colour.

Inhibition of yeast growth. The antibiotic Actidione (5) was incorporated in all primary isolation plates at a level of 5 p.p.m. (4). The addition of this substance was found, by Millis, to inhibit most yeasts, whilst leaving the lactic acid bacteria unaffected. Although some of the yeasts encountered in fermenting apple juices were resistant to its action at all levels, this method was found to be more satisfactory than differential centrifugation (6).

Methods of incubation. All plates were incubated at 25° C. in evacuated jars containing a mixed solution of pyrogallol and sodium carbonate. Similar treatment of the liquid media produced a slightly enhanced growth, but, for routine purposes, the use of an evacuated jar was not necessary.

Two distinct malate-splitting organisms were isolated by the use of the preceding methods, a rod and a coccus.

Description of the Organisms :
Morphological and Cultural Characteristics.

Appearance of the organisms in a fixed and Gram-stained film. The rods after 7 days at 25° C. had an average size of 4–5 μ × 0·6 μ and occurred in palisades, pairs and sometimes chains. A few long forms of 20–40 μ were also apparent. The coccus, after the same length of time, had an average size of 0·75 μ × 0·25 μ and occurred mainly in clumps, though pairs and short chains were observed less frequently. Both organisms were Gram-positive, though the rods showed a tendency to lose this characteristic in older cultures. The presence of capsules and endospores was not demonstrated in either culture, neither was motility observed.

Colonial Characteristics. The rods gave colonies with a maximum diameter of 1·8 mm. after anaerobic incubation for 7 days, at which time they were roughly circular with an irregular surface. In elevation, the colonies presented a raised central portion surrounded by a flatter marginal area. The texture was butyrous and the colour yellowish, becoming buff in older cultures. The colonies of the coccus took 10 days to reach a maximum diameter of 1·5 mm. and produced an uncharacteristic smooth, circular growth with an entire margin and convex elevation. The colour was greyish-white and the texture butyrous, but not as solid as the growth of the rod.

Growth in liquid AJYE at 25° C. The rods produced a typical " silky " turbidity in the medium on reaching maximum growth, and after 2–3 days this was accompanied by a steady evolution of gas. A characteristic of this organism was the production of only a very slight sediment, the majority of cells remaining in suspension even after 26 days' incubation. The coccus differed quite distinctly from the rod, for the main growth occurred in a solid non-viscous mass at the base of the tube. This deposit could be dislodged by agitation, to produce a mass of suspended floccules accompanied by a turbidity of medium intensity. No gas was observed.

Physiological Characteristics.

A summarised version of these features appears in a table below.

TABLE I.

PHYSIOLOGICAL CHARACTERISTICS.

Organism	Oxygen relations	Optimum pH range	pH Min. : Max.	Optimum temp. (° C.)	Thermal death point	Alcohol tolerance	SO_2 tolerance
Rod	Facultative anaerobe	4·5–5·6	3·8 : 6·8	25–30	Survival 2½ min. at 60° C.	Strong growth at 8% V/V and trace growth up to 12%	No growth at 200 p.p.m. Retarded growth at 100 p.p.m.
Coccus	Facultative anaerobe	4·5–5·2	3·5 : 6·4	25–30	Survival 10 min. at 60° C.	Strong growth at 6% V/V and trace growth up to 12%	No growth at 200 p.p.m. Retarded growth at 150 p.p.m.

Oxygen relations. Although both organisms were classified as facultative anaerobes, the coccus was more tolerant of oxygen than the rod. This was shown in a stab culture where the coccus was able to grow throughout the length of the stab, whereas the growth of the rod, under similar conditions, reached to 1 cm. below the surface of the agar. Relation to pH : the optimum range for growth was very similar for the rod and coccus, but the minimum and maximum values for growth differed for the two organisms. Optimum temperature range : this was the same for the rod and coccus. Thermal death point : the coccus was found to be more resistant than the rod at all times and temperatures. Alcohol tolerance : the growth of the rod was less readily retarded than that of the coccus. Sulphur dioxide tolerance : neither organism was able to grow at 200 p.p.m. and both were greatly retarded at lower concentrations than this.

The significance of the physiological characteristics of these organisms will be discussed later, in relation to the control and application of the malo-lactic fermentation.

Biochemical Activities.

Carbohydrate fermentations. These tests were carried out using a basal medium containing casein hydrolysate, yeast extract and mineral salts with bromo-cresol green indicator (pH 4·8). Sterile-filtered solutions of carbohydrates were added to obtain a final concentration of 2%. The results of these fermentations are listed in Table II :—

TABLE II.

Sugars and Alcohols	Rod	Coccus	Key
Glucose	A	a	
Fructose	A	A	
Maltose	A	—	
Sucrose	—	—	A = strong production of acid.
Galactose	a	—	
Lactose	—	—	
Arabinose	A	A	
Xylose	A	—	
Raffinose	—	—	a = weak production of acid
Rhamnose	—	—	
Mannitol	—	—	
Sorbitol	—	—	
Dulcitol	—	—	

All tests contained inverted Durham tubes but no gas production was demonstrated. The products of the fermentation of glucose and arabinose were determined chromatographically and the principal product was shown to be lactic acid.

Type of Fermentation. The fixed and volatile acids, produced by the fermentation of glucose, were determined by titration. Both organisms proved to be heterofermentative with the following molecular ratios of fixed to volatile acid : the value for the rod was 100:17·9 and for the coccus 100:17·5.

Other Biochemical characteristics. These are tabulated in Table III.

Although the coccus was unable to attack lactose, growth was sustained in milk, probably by the utilisation of the citrate present (7). Both organisms were tested for the utilisation of citrate but only the coccus was found able to use this acid as a source of energy, with the production of volatile acid.

TABLE III.

	Rod	Coccus
Catalase reaction	Negative	Negative
Indole production	Negative	Negative
Reduction of nitrate	Negative	Negative
Production of H_2S	Negative	Negative
Production of NH_3	Negative	Negative
Liquefaction of gelatin	Negative	Negative
Acetyl methyl carbinol production ..	Negative	Negative
Growth in litmus milk	Very feeble	Slight with a slow clot formation and reduction of the indicator.

Classification.

Both organisms have been identified as members of the family *Lactobacteriaceae* Orla-Jensen in view of the following characteristics : Gram-positive, non-motile, facultative anaerobes, non-reduction of nitrate.

The Rod.

This organism has been assigned to the sub-genus *Betabacterium* Orla-Jensen within the genus *Lactobacillus* Beijerinck (8) because of its heterofermentation of glucose and optimum temperature range of 25–30° C. Of the organisms within this sub-genus, *Lactobacillus brevis* Orla-Jensen was the one the cider isolate most nearly resembled, but comparative tests with a known strain of this bacterium* and the rod from cider, revealed that they differed in several details. The isolate from cider had a much more irregular colony than the named species and exhibited a less vigorous growth under all conditions. The most marked difference between the two organisms was the ability of the former to remain suspended in liquid medium (AJYE) in contrast to *L.brevis*, which gave rise to a heavy deposit and clear supernatant fluid in 2–3 days.

The Coccus.

The heterofermentation of glucose and the tendency to form cocco-bacilli in short chains placed the coccus within the genus *Leuconostoc* Van Tieghem (8). *Leuconostoc citrovorum* Hammer was the species which the coccus resembled most closely on the basis of its failure to produce slime or to ferment sucrose. The coccus from cider was able to utilise citric acid as a sole source of carbon, in contrast to *Leuc.citrovorum*, which requires small amounts of glucose, in addition, to initiate growth (7). The cider isolate also differed from *Leuconostoc citrovorum* because it fermented arabinose but not lactose.

* Culture obtained from the National Collection of Industrial Bacteria.

The coccus from cider appeared to be more closely related to *Bacterium gracile*, which has recently been stated to be a possible member of the genus *Leuconostoc* (9). These organisms were both capable of utilising malic and citric acid but differed to some extent in their fermentation of sugars. The coccus from cider fermented glucose, fructose and arabinose while, according to Muller-Thurgau and Osterwalder, *B.gracile* fermented glucose, fructose and galactose (1). The optimum temperatures for *B.gracile* and the species of the genus *Leuconostoc* all came within the range 20°–26° C., differing from the cider isolate, which had an optimum between 25° and 30° C.

Neither of these cider isolates has been assigned to a species as yet. It is considered that a wider range of organisms from ciders should be investigated and compared with established species, grown under the same cultural conditions, before a true identification can be made.

Application and Control of the Malo-lactic Fermentation.

Although only two organisms have been examined in detail, present work indicates that the bacterial breakdown of malic acid may be very much more general in English cider-making practice than is at present realised. Indeed, Müller-Thurgau and Osterwalder (1) indicated how widespread this type of organism was in wines, ciders and perries.

This fermentation in ciders and perries may be advantageous or otherwise according to conditions. In some seasons, when cider apple crops are poor, it becomes necessary to make more use of culinary and dessert fruit. In such an event, a malo-lactic fermentation offers a means of utilising high-acid fruit, by reducing the acidity without markedly changing the flavour. This is not without its disadvantages, however, because pure culture experiments have shown that some fruity flavour and aroma may be lost as a result. This loss of flavour is partially counterbalanced by a more mature and vinous character.

A disadvantage of spontaneous malo-lactic fermentation, particularly in the low-acid, sweet ciders of Somerset and Devon, is that a reduction of acidity may give rise to conditions suitable for the development of the cider sickness organism *Saccharomonas anaerobium*. To prevent such occurrences, some measure of control is desirable. It has been shown in an earlier section that malo-lactic organisms are susceptible to the action of sulphur dioxide and, also, that they grow readily only within a limited temperature range. Addition of sulphur dioxide to the juice gives some protection against the activity of these organisms, but more satisfactory control may be attained either by the use of flash pasteurisation or by fermentation at a temperature unfavourable to the development of the bacteria.

Where bacterial deacidification is required, it is best allowed to proceed in a *dry* cider, either spontaneously or by the addition of a pure culture of the organisms. The reasons for this are as follows : (1) the absence of fermentable sugar eliminates the appearance of sickness, even though the acid level may be favourable ; (2) there is no danger of sugar fermentation by the malo-lactic bacteria, with the subsequent development of off-flavours ; (3) the presence of yeast autolysis products may provide essential growth factors for the bacteria.

It should be noted that some of the high acid juices may not support growth of the malo-lactic organisms because of their very low pH values. In such circumstances it may be necessary to adjust the pH to a suitable level (see Table I) in order to facilitate growth after inoculation. This, too, supports the practice of addition of active cultures at dryness, for should it be necessary to adjust the pH, then there is less danger of provoking disorders at dryness than in the juice stage.

Summary.

Two malate-decomposing organisms, one a rod, the other a coccus, have been isolated from cider. The media and methods of isolation are described and the morphological, cultural, biochemical and physiological characteristics of the bacteria are given. Neither organism has been assigned to a species, but it is considered that the rod belongs to the sub-genus *Betabacterium*, and the coccus is an atypical member of the genus *Leuconostoc*.

Acknowledgments.

The author wishes to acknowledge the interest shown and help given by Dr. A. Pollard during this work, and the assistance of Mr. J. D. Phillips in carrying out the chromatographic analyses.

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AN INVESTIGATION OF LEAD ARSENATE SPRAY RESIDUES ON APPLES AND IN THEIR PRODUCTS.

By C. F. TIMBERLAKE and A. POLLARD.

Proposals for more stringent regulations of the amounts of lead and arsenic in fruit juices and ciders have called for further information on the amounts that occur as a result of contamination by spray residues. During the 1951-52 season, an investigation has been made of the amount of residue remaining on apples which were sprayed with lead arsenate and its fate during processing to juice, cider and pectin.

Lead arsenate is used at 0·2% concentration to control codling moth on apples. Two applications may be given ; the normal recommendation is to spray during the second half of June and again up to mid-July. Cider varieties of apple are seldom sprayed with lead arsenate, for economic reasons, but a cider variety, Dabinett, was included in these tests to determine the fate of the spray residue during cider-making.

Applications of a proprietary brand of commercial lead arsenate at 0·2% concentration (2 lbs. 100 gallons), with no further addition, were made later than normal as follows :—

Dabinett .. .. 17 July and 30 July, 1951.
Bramley .. .. 19 July and 3 August, 1951.

Deposits were determined on samples of fruit taken immediately after spraying, and at harvest, that is, 79 days later than the second spraying of Dabinett, and 73 days later than the second spraying of Bramley. After harvest, large batches of fruit were washed with water and acid, and lead and arsenic followed in the processing of unwashed and washed Dabinett for juice, cider and pomace and of unwashed and washed Bramley for juice. The lead and arsenic contents of pectin made from pomace obtained from sprayed fruit were also determined.

Experimental.

Materials.

Dabinett.

Spraying.

Six adjacent trees in the same row were sprayed on 17/7/51. Samples of fruit were taken immediately before and after spraying. Three trees were sprayed a second time on 30 7 51 and samples of sprayed fruit taken as before. Further samples were taken from the six trees on 17/10/51, immediately prior to harvesting the main bulk of apples. As a control, samples of unsprayed fruit were taken from a row at some distance away in an adjoining plot.

Washing Treatments.

Bulks of fruit of at least 2 cwt. were collected from each spray treatment and designated as follows :—

A : unsprayed fruit.
B : fruit sprayed once.
C : fruit sprayed twice.

Batches B and C were divided into three portions of 30–40 kg. each, which were, respectively, left unwashed, washed with water or washed with acid. The actual treatments given were as follows :—

A/2 : unsprayed fruit, water washed.
B/3 : fruit sprayed once, acid washed.
C/3 : fruit sprayed twice, acid washed.
B/2 : fruit sprayed once, water washed.
C/2 : fruit sprayed twice, water washed.
B/1 : fruit sprayed once, unwashed.
C/1 : fruit sprayed twice, unwashed.

Water wash : Each batch of fruit was thoroughly washed in a large drum and then sprayed on wooden racks with a water jet.

Acid wash : The apples were immersed in a porcelain sink in 1% HCl (125 mls. conc. HCl per gallon of water) for 3 minutes at 54–55° C. (130° F.), the acid solution being previously brought to 65–70° C. by steam injection. The acid was then drained off and the fruit washed with water as before.

Representative samples of fruit from each batch after washing were taken for analysis.

Milling and Pressing.

The batches of fruit were milled immediately and pressed in a small experimental cider press. The order of milling and pressing followed the order of treatments given above which was designed to give the least carry-over of spray residue from one pressing to another. The yields of juice and pomace for each treatment were noted and samples taken for analysis.

Preparation of Ciders.

A one-gallon sample of juice from each treatment was allowed to ferment to dryness in a glass jar. When dry, the cider was racked and in four cases, was filtered through a Carlson E.K. filter and bottled. In the other three cases, the cider was not filtered, but bottled directly after racking, as analyses indicated that some contamination with lead was occurring during filtration.

Bramley.

Spraying.

Twelve trees in the same row were sprayed on 19/7/51 and samples of fruit were taken for analysis before and after spraying. Six trees were sprayed a second time on 3/8/51 and samples of sprayed fruit taken as before. Further samples were taken from the twelve trees on 15/10/51, immediately prior to harvesting the main bulk of apples.

Washing, Milling and Pressing.

The washing treatments, milling and pressing were as already described for Dabinett.

Methods.

Sampling Methods.

Fruit sampling : Quarters were cut from about 20 apples in each sample, weighed and peeled. The peels were dried for analysis in 400 ml. beakers and subsequently digested in the same beakers. Additional samples of peeled and unpeeled apple sections were dried and preserved in cellophane bags for later analysis.

Juice sampling : Juices were sampled immediately after pressing and stored in conical flasks for analysis.

Pomace sampling : Samples of 1,500 g. of wet pomace were dried in a steam oven on aluminium trays lined with grease-proof paper. The pomace from each treatment, when dry, was ground by hand in a large mortar, mixed and stored in Kilner jars. Four samples, each about 40 g., of the dried pomace from each treatment, were selected, and 10 g. from each sample taken for analysis.

The moisture content of the pomace was determined by drying 5 g. samples in the Carter-Simon Rapid Moisture Tester at 135° C. for 15 minutes.

Cider sampling : Samples of the ciders were taken in conical flasks for analysis after racking and again after filtering.

Chemical Methods.

Acid digestion : Fruit and pomace samples were digested with a mixture of nitric and perchloric acids in 400 ml. beakers, covered with watchglasses, on a hot plate. Separate samples of juice and cider were digested with nitric and perchloric acids in conical flasks on a hot plate for the determination of lead, and with a mixture of nitric, perchloric and sulphuric acids in 300 ml. Kjeldahl flasks for the determination of arsenic.

Determination of lead : Lead was determined in the digested solutions using dithizone, by the method of Sandell (1).

Determination of arsenic : Arsenic was determined in fruit, pomace and juice by the method of Levvy (2), in which arsenic is separated from the digested solution as arsine, passed into silver nitrate solution, and the arsenite formed titrated with dilute iodine. The arsenic in cider, where the general level was lower, was determined by means of the Gutzeit Test (3).

An additional determination was made on Bramley fruit, in which the spray residue was dissolved off the surface of 1 kg. of apples with a mixture of dilute sodium hydroxide and sodium oleate, according to the A.O.A.C. rapid method of analysis (4), stated to give a 95% efficiency.

Control Determinations.

Control digestions were carried out on samples of unsprayed fruit and on juice and pomace prepared from it, with and without the addition of known amounts of lead (as lead nitrate) and arsenic (as sodium arsenite). The additions of lead and arsenic were made over the appropriate ranges in p.p.m. for each material and recoveries were as follows :—

Fruit ;	lead 98–104%,	arsenic 97–105%.
Juice ;	lead 93–96%,	arsenic 95–105%.
Pomace ;	lead 97–102%,	arsenic 99–107%.

Results.

Lead and arsenic in fruit, juice, pomace and cider.

The results are shown in the following tables :—

Table I. Analyses of Dabinett fruit at time of spraying and at harvest.

Table II. Analyses of Bramley fruit at time of spraying and at harvest.
Table III. Analyses of Dabinett fruit, juice, pomace and cider.
Table IV. Analyses of Bramley fruit and juice at time of pressing.
Table V. The distribution of lead and arsenic between Dabinett juice and pomace.
Table VI. The lead and arsenic contents of an "average" apple at time of spraying and at harvest.

In the above tables, lead and arsenic are expressed in terms of the elements (Pb and As). The figures are expressed as p.p.m. of the fresh weights of the materials, except where otherwise stated.

The more important data from these tables are summarised below.

TABLE I.

DABINETT.

ANALYSES OF FRUIT AT TIME OF SPRAYING AND AT HARVEST.

Fruit	Sampling Date	No. of samples	Lead p.p.m.		Arsenic p.p.m. (as *As*)	
			Range	Mean	Range	Mean
Unsprayed	16/7/51 Before spraying ..	1		0·10		<0·04
	17/10/51 Control sample at harvest ..	4	0·08–0·17	0·11		<0·04
Sprayed once	17/7/51 After spraying ..	3	9·1–10·6	10·1	3·6– 4·5	4·1
	17/10/51 At harvest ..	3	1·79–2·13	1·93	0·48–0·61	0·54
Sprayed twice	30/7/51 After spraying ..	4	12·7–15·0	13·8	4·8 –5·8	5·4
	17/10/51 At harvest ..	11	1·88–4·42	3·22	0·35–1·33	0·87

TABLE II.

BRAMLEY.

ANALYSES OF FRUIT AT TIME OF SPRAYING AND AT HARVEST.

Fruit	Sampling Date	No. of samples	Lead p.p.m.		Arsenic p.p.m. (as *As*)	
			Range	Mean	Range	Mean
Unsprayed	18/7/51 Before spraying ..	2	0·17–0·33	0·25		<0·04
Sprayed once	19/7/51 After spraying ..	4	8·1 –8·9	8·46	2·62–2·95	2·76
	15/10/51 At harvest ..	4	1·03–1·64	1·44	0·29–0·57	0·47
Sprayed twice	3/8/51 After spraying ..	4	7·2 –9·2	8·45	2·4 –3·2	2·88
	15/10/51 At harvest ..	9	1·73–3·80	2·65 2·54*	0·50–1·19	0·86 0·84*

*Determined by Rapid Method (Ref. 4).

TABLE III.

DABINETT.

ANALYSES OF FRUIT*, JUICE, DRIED POMACE AND CIDER.

Code	Treatment	Fruit*		Juice		Pomace (on moisture free basis)		Cider	
		Lead p.p.m.	Arsenic (as *As*) p.p.m.	Lead p.p.m.	Arsenic (as *As*) p.p.m.	Lead p.p.m.	Arsenic (as *As*) p.p.m.	Lead p.p.m.	Arsenic (as *As*) p.p.m.
A/2	Unsprayed apples water washed	0·28	—	0·09	<0·01	3·2	<0·1	0·04	<0·01
B/1	Once sprayed apples unwashed	2·02	0·43	0·18	0·10	28·3	5·3	0·17	0·04
B/2	Once sprayed apples water washed	2·27	0·43	0·16	0·08	32·7	5·5	0·13	0·05
B/3	Once sprayed apples .. acid washed	0·93	0·32	0·12	0·08	12·0	3·6	0·06	0·04
C/1	Twice sprayed apples unwashed	3·93	0·77	0·16	0·13	57·2	10·1	0·08	0·05
C/2	Twice sprayed apples water washed	3·36	0·66	0·10	0·13	49·6	8·5	0·05	0·04
C/3	Twice sprayed apples acid washed	1·47	0·50	0·11	0·12	20·0	6·0	0·05	0·05

*Derived from the summation of juice and pomace figures.
Fruit .. .. figures expressed as p.p.m. of fresh weight.
Juice and Cider .. ,, ,, ,, ,, ,, liquid product.
Pomace .. .. ,, ,, ,, ,, on moisture free basis.

TABLE IV.

BRAMLEY.

ANALYSES OF FRUIT AND JUICE AT TIME OF PRESSING. 19/11/51.

Code	Treatment	Apples			Juice	
		wt. of sample g.	Lead p.p.m.	Arsenic (as *As*) p.p.m.	Lead p.p.m.	Arsenic (as *As*) p.p.m.
B/1	Once sprayed apples .. unwashed	107·0 79·4	1·59 2·54	0·49 0·70	0·19	0·11
B/2	Once sprayed apples .. water washed	85·8 88·7	2·31 1·52	0·77 0·34	0·20	0·10
B/3	Once sprayed apples .. acid washed	115·6 102·1	0·54 0·25	0·17 —	0·17	0·04
C/1	Twice sprayed apples .. unwashed	93·2 100·8	4·97 3·15	1·55 0·85	0·23	0·21
C/2	Twice sprayed apples .. water washed	88·3 96·0	4·36 3·00	1·38 1·08	0·21	0·23
C/3	Twice sprayed apples .. acid washed	124·9 102·5	0·85 0·42	0·35 —	0·16	0·05

Apples — figures expressed as p.p.m. of fresh weight.
Juice — ,, ,, ,, ,, ,, liquid product.

TABLE V.

DABINETT.

DISTRIBUTION OF LEAD AND ARSENIC BETWEEN JUICE AND POMACE.

Treatment	% Total lead in juice	% Total lead in pomace	% Arsenic in juice	% Arsenic in pomace
A/2	23·2	76·8	—	—
B/1	6·4	93·6	17·8	82·2
B/2	4·9	95·1	14·3	85·7
B/3	9·3	90·7	19·1	80·9
C/1	2·9	97·1	12·1	87·9
C/2	2·2	97·8	14·7	85·3
C/3	5·5	94·5	17·2	82·8

TABLE VI.

THE LEAD AND ARSENIC CONTENTS OF AN "AVERAGE" APPLE AT TIME OF SPRAYING AND AT HARVEST.

Variety	Spray Treatment	Date of Sampling		Average wt. of apple in g.	Average lead p.p.m.	Average arsenic p.p.m.	µg. lead on average apple	µg. arsenic on average apple
Dabinett	Sprayed once	17/7/51	After spraying ..	10·4	10·1	4·07	105	42
		17/10/51	At harvest ..	65·7	1·93	0·54	127	36
	Sprayed twice	30/7/51	After spraying ..	19·0	13·8	5·37	262	102
		17/10/51	At harvest ..	68·8	3·22	0·87	222	60
Bramley	Sprayed once	19/7/51	After spraying ..	26·5	8·46	2·76	224	73
		15/10/51	At harvest ..	115·8	1·44	0·47	167	54
	Sprayed twice	3/8/51	After spraying ..	54·3	8·45	2·88	459	156
		15/10/51	At harvest ..	117·7	2·65	0·86	313	101

Dabinett.

Fruit (Table I).

The lead content of fruit sampled immediately after the first spraying varied from 9·1–10·6 p.p.m. (mean 10·1 p.p.m.), and the arsenic content from 3·6–4·5 p.p.m. (mean 4·1 p.p.m.). The figures obtained for fruit sampled immediately after the second spraying were higher; lead varied from 12·7–15·0 p.p.m. (mean 13·8 p.p.m.) and arsenic varied from 4·8–5·8 p.p.m. (mean 5·4 p.p.m.). At harvest the levels of lead and arsenic on the fruit were considerably reduced. Once-sprayed fruit then contained 1·8–2·1 p.p.m. (mean 1·9 p.p.m.) of lead and 0·5–0·6 p.p.m. (mean 0·5 p.p.m.) of arsenic; twice-sprayed fruit contained 1·9–4·4 p.p.m. (mean 3·2 p.p.m.) of lead, and 0·6–1·3 p.p.m. (mean 0·9 p.p.m.) of arsenic. The analyses of lead and arsenic on the fruit at harvest (Table I) show variations between samples, but the means shown above are in good agreement with figures derived by summation of those for juice and pomace viz., once-sprayed fruit, 2·0 p.p.m. lead, 0·4 p.p.m. arsenic; twice-sprayed fruit, 3·9 p.p.m. lead, 0·8 p.p.m. arsenic (Table III). In view of the variation in the amounts of residue on individual fruits, an estimate based on the figures for juice and pomace, derived from a large bulk of fruit, offers a more reliable indication of the average level of lead and arsenic on the fruit. The effects of washing treatments on the lead and arsenic levels of the fruit have therefore been estimated from the juice and pomace figures in Table III.

Effects of washing treatments on fruit: Water washing produced no loss of lead and arsenic from once-sprayed fruit, but gave a slight loss of lead and arsenic from twice-sprayed fruit.

Acid washing was more efficient and removed one-half of the lead and one-quarter of the arsenic from once-sprayed fruit, and two-thirds of the lead and one-third of the arsenic from twice-sprayed fruit.

Juice (Table III).

The juice obtained from each of the pressings of sprayed fruit contained less than 10% of the lead and less than 20% of the arsenic present on the fruit (Table V). The lead contents of the juices varied from 0·10–0·18 p.p.m. and were not consistently affected by the amount of residue on the fruit. The arsenic content of the juices varied from 0·08–0·13 p.p.m., and were highest in the juices prepared from twice-sprayed fruit.

Acid washing of the fruit slightly reduced the lead content of juice, but had little influence on the arsenic content of the juice.

Ciders (Table III).

The lead and arsenic contents of the ciders were in each case lower than those of the freshly-expressed juices, thus indicating the removal of these elements, to some extent, in the lees during fermentation. Lead in the racked cider varied from 0·05–0·17 p.p.m., and was highest in ciders prepared from once-sprayed fruit. Arsenic in the racked ciders varied from 0·04–0·05 p.p.m. Acid washing of the fruit was effective in producing ciders with a lead content only slightly greater than that of cider prepared from unsprayed fruit, but had little influence on the arsenic content of the cider.

Pomace (Table III).

The greater part of the lead and arsenic residue on the fruit was localised in the pomace. Lead in pomace from sprayed fruit was reduced only by acid washing of the fruit, which brought it down to less than 50% of the amount present in pomace from unwashed fruit. Arsenic was also reduced by acid washing but less effectively, to 60–70% of the amount present in pomace from unwashed fruit.

Bramley.

Fruit (Table II).

The lead content of fruit sampled immediately after the first spraying varied from 8·1–8·9 p.p.m. (mean 8·5 p.p.m.) and the arsenic content from 2·6–3·0 p.p.m. (mean 2·8 p.p.m.). Fruit sampled immediately after the second spraying had the following contents: lead 7·2–9·2 p.p.m. (mean 8·5 p.p.m.), arsenic 2·4–3·2 p.p.m. (mean 2·9 p.p.m.).

At harvest once-sprayed fruit contained 1·0–1·6 p.p.m. (mean 1·4 p.p.m.) of lead: 0·3–0·6 p.p.m. (mean 0·5 p.p.m.) of arsenic: twice-sprayed fruit contained 1·7–3·8 p.p.m. (mean 2·7 p.p.m.) of lead, and 0·5–1·2 p.p.m. (mean 0·9 p.p.m.) of arsenic.

Thus at the time of spraying, levels of lead and arsenic per given weight of fruit were lower in Bramley than in the smaller fruit of Dabinett. At harvest, the two varieties contained similar amounts of spray residue.

Juice (Table IV).

The lead contents of the juices varied from 0·16–0·23 p.p.m. and the arsenic contents from 0·04–0·23 p.p.m. according to the washing treatment. The lead and arsenic contents of Bramley juice from unwashed fruit were both slightly higher than those of Dabinett juice.

Effects of washing treatments on fruit and juice.

Water : water washing was, as with Dabinett, inefficient in reducing lead and arsenic in fruit and juice.

Acid : acid washing was again efficient in removing lead and arsenic from the fruit.

Lead in juice was slightly reduced, and arsenic in juice removed to the extent of 60–70%. Thus, washing the fruit with acid was more effective in removing arsenic from Bramley than from Dabinett.

Lead and arsenic in pectin.

Analyses of the pressed pomace have shown that the greater part of the lead and arsenic on the fruit remains in the pomace after juice extraction. Since dried apple pomace is utilised largely in the manufacture of pectin, the distribution of lead and arsenic occurring during the process of pectin extraction is of considerable importance, and it has therefore been followed in the extraction of pectin from samples of pomace obtained from sprayed and unsprayed Bramley fruit.

Extraction of pectin.

Samples of uncontaminated and contaminated pomace were reduced to a fine powder in a malt mill and 50 gm. of each leached with cold water (500 mls.) for 30 minutes. After filtration through muslin under pressure, the residue was extracted three times with dilute HCl at pH 2·8–3·0 for 90 minutes at 80–85° C. The combined liquid pectin extracts from each sample, obtained by filtration through muslin under pressure, were centrifuged and made up to a definite volume from which aliquots were taken for the analyses of lead and arsenic. The remainders of the liquid pectin extracts were precipitated with two volumes of acetone. The solid pectin was collected, re-precipitated with acetone, soaked in pure acetone, and finally dried in a vacuum desiccator. The exhausted pomace residues were also dried for analysis. The yields of pectin (based on the Ca pectate values for the liquid extracts) were 8% of the weights of pomace.

The results are shown in the following tables.

Table VII. Distribution of lead and arsenic during extraction of pectin.

Table VIII. The lead and arsenic contents of pomace, pectin, and pomace residue (as p.p.m.).

TABLE VII.

DISTRIBUTION OF LEAD AND ARSENIC DURING EXTRACTION OF PECTIN.

Material analysed	Unsprayed pomace (50 g.)			Sprayed pomace (50 g.)			
	Lead		Arsenic	Lead		Arsenic	
	mg.	% of total	mg.	mg.	% of total	mg.	% of total
Cold water extract..	0·013	3	NIL	0·143	3	0·200	17
Pectin solution ..	0·135	26	NIL	0·450	10	0·560	46
Exhausted residue..	0·366	71	NIL	3·841	87	0·443	37

TABLE VIII.

LEAD AND ARSENIC IN POMACE AND PECTIN (AS P.P.M.).

Material analysed	Unsprayed pomace			Sprayed pomace		
	Ca pectate value	Lead (p.p.m.)	Arsenic (p.p.m.)	Ca pectate value	Lead (p.p.m.)	Arsenic (p.p.m.)
Original pomace ..		8·2	$<0·2$		106	26
Pectin solution ..	0·31%	0·1	$<0·03$	0·29%	0·3	0·4
Solid pectin ..	72·5%	34	$<1·0$	88·0%	77	$<2·0$
Exhausted residue..		16·0	$<0·1$		177	20

The results are summarised as follows :—

Cold water leaching :—This removed an appreciable quantity of arsenic but very little of the total lead from the contaminated pomace.

Pectin solution :—The pectin solution contained slightly more arsenic than lead, and retained almost 50% of the total arsenic and 10% of the total lead present in the contaminated pomace.

Exhausted pomace residue :—The exhausted pomace residue contained nine times more lead than arsenic, and nearly 90% of the total lead and 40% of the total arsenic present in the contaminated pomace.

Solid pectin :—The solid pectin obtained by precipitation of the pectin solution with acetone retained most of the lead (100% for uncontaminated and 80% for contaminated pomace), but less than 2% of the arsenic present in the pectin solution.

Discussion.

Lead arsenate is widely used as an insecticide for the control of codling moth on apples, and the spray residue remaining on the harvested fruit and in its products may cause the proposed levels of lead and arsenic permitted in foodstuffs to be exceeded. This investigation was undertaken to determine the amounts of lead and arsenic occurring in apples and in apple juice, cider and pectin, due to the spray residue retained by the fruit. Shaw (5) found that apples and pears may be safely sprayed up to six weeks before picking early varieties or up to mid-July in the case of later varieties. Little of the lead arsenate was lost by weathering (6), attenuation of the deposit by growth of the fruit bringing the arsenic residue within the tolerance. In the present trials, the considerable reduction in the lead and arsenic content of the fruit (as p.p.m.) between spraying and harvesting may be attributed largely to the growth of the fruit during this period. There was however an indication of some loss of lead and arsenic on the fruit due to weathering. Table VI shows that the lead and arsenic contents, calculated for an " average " apple, are, with one exception, lower at harvest than immediately after spraying. The rainfall (9″) over this period, differed only slightly from the average (10″).

Dilute hydrochloric acid (1%) has been used in commercial practice to wash sprayed apples, and its effect in reducing the quantity of residue remaining on the fruit and in its products has been studied in the present trials. Brydon and Hall (7) found that best results were obtained with the acid at a temperature of 80–90° F. followed by thorough washing with water. Liability to injury was increased as the bath temperature increased above 100° F., and as the acid concentration was raised to 1·5%. Atkinson and Strachan (8) used a washer in which 1% HCl was flooded over the fruit at a temperature approximating 130° F., followed by a cold water wash. They found that this wash may slightly crack the skins but regarded the injury as unimportant, as the apples were ground shortly after leaving the washer. In the present tests, the fruit was washed for three minutes in 1% hydrochloric acid at approximately 130° F. (54–55° C.), followed by thorough washing with water. Fruit injury appeared to be very slight but was not studied in detail as the washed fruit was processed immediately into juice.

Acid washing of the fruit was effective in removing more than half the lead and one-quarter of the arsenic on Dabinett. With Bramley the results were less conclusive since analyses were confined to fruit samples where the sampling errors were greater. The analyses of sections of washed fruit, however, indicated that a considerable reduction in lead and arsenic content was again obtained. Acid washing of the fruit had a pronounced effect in reducing the arsenic content of Bramley juice. It also effected slight reductions in the lead content of Bramley juice, and of Dabinett juice and cider. Water washing alone was ineffective in reducing the lead and arsenic contents of either fruit, juice or cider.

The relative proportion of arsenic to lead in the pressed juices was always greater than that found on the fruit, i.e. arsenic was the more readily soluble in the juice. Thus although the amount of lead on the fruit was greater than the amount of arsenic, the arsenic contents of the juices approached very closely or even exceeded the lead contents. A similar effect was observed with a pectin solution which retained slightly more arsenic than lead, although the contaminated pomace from which it was made contained considerably more lead than arsenic.

At the time of writing, the limits for lead and arsenic which have been recommended by the Metallic Contamination Sub-Committee of the Food Standards Committee are as follows (9) :—

	Lead	Arsenic (as As)
Juice	0·5 p.p.m.	0·1 p.p.m.
Cider	0·5 p.p.m.	0·2 p.p.m.
Liquid Pectin	10 p.p.m.	2·0 p.p.m.
Solid Pectin	" under consideration "	2·0 p.p.m.
" Other Foods " (including Fruit)	2·0 p.p.m.	1·0 p.p.m.

In view of these recommendations the following observations may be made :—

Fruit :—Sprayed fruit may be above the limits for lead, unless acid washed, and may be near or above the limits for arsenic in individual fruits (Tables I and II).

Juice :—The freshly-expressed juices should be within the limits for lead whether the fruit is acid washed or not, but may be above the limit for arsenic when the fruit is unwashed. Acid washing of the fruit may bring the juices within the limit for arsenic (Tables III and IV).

Cider :—Cider should be within the limits for both lead and arsenic whether the fruit is acid washed or not (Table III).

Liquid pectin : –Concentration of the pectin solutions to 3% (based on the calcium pectate values in Table VIII), would give the following figures :

	Lead	Arsenic
Pectin prepared from pomace from unsprayed fruit ..	1·0 p.p.m.	< 0·3 p.p.m.
Pectin prepared from pomace from sprayed fruit ..	3·0 p.p.m.	4·0 p.p.m.

Thus, lead would be below the limit even if a much stronger pectin solution were prepared. Arsenic, however, would be above the limit.

Conclusions.

The results summarised above indicate that the problems raised by the presence of lead arsenate residues on apples vary according to the use to which the fruit is put. If it is to be consumed in the fresh state, the tolerance for lead is more likely to be exceeded than the tolerance for arsenic. The juice prepared from the fruit or pectin extracts prepared from the pomace may, however, contain excessive amounts of arsenic.

These findings are based on the result of two spray applications made a fortnight later than usual. Earlier spraying would tend to give smaller amounts of spray residue than were found in these tests, but examination of the data for fruit sprayed once only in mid-July shows that the fruit can still retain at harvest an amount of lead which approaches the limit or, on small fruit, exceeds it. The juice prepared from such fruit can still exceed the limit permitted for arsenic.

The amount of residue left on the fruit can be reduced by the use of acid washes : in the absence of such treatment it must be concluded that the use of lead arsenate sprays as late as mid-July leaves little latitude if the levels of lead and arsenic are to be kept below the present permitted limits.

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FACTORS AFFECTING THE QUALITY AND CHEMICAL CONSTITUENTS OF STRAWBERRIES : EFFECTS OF MANURIAL TREATMENTS.

By MARGARET E. KIESER, A. POLLARD and C. F. TIMBERLAKE.

Strawberries are largely consumed as dessert fruit, therefore any estimate of their quality must include an assessment of appearance, texture and flavour as well as a chemical analysis of components such as ascorbic acid, sugar and titratable acid. For jam-making, the pectin content is of interest even though additions of this substance are almost invariably made. Ideally, strawberries of high quality should have good flavour and aroma, an attractive appearance, even size, tender texture when ripe, high vitamin C content, high sugar and acid content and, for jam-making in particular, a high content of good quality pectin. Low-grade fruit, on the contrary, will lack flavour and aroma, be unattractive or uneven in colour, irregular in size and shape, tough, soft, or uneven in texture, low in ascorbic acid content, and either low in sugar or in both sugar and acid, thus having a watery and insipid flavour, while pectin may be low in quantity or quality.

An attempt was made in the experiments described below to assess all the above factors with the exception of size and pectin quality. A series of samples of one variety of strawberry was examined during the 1951 and 1952 seasons, to determine the effect of manurial treatments upon chemical composition and fruit quality.

Experimental.

Material and Treatments.

Plots. Plants of the variety Auchincruive Climax were set out in 1950 as runners on plots at Long Ashton. The plants were de-blossomed in 1950 and no harvesting was begun until July, 1951.

The soil of these plots is a fine sandy silt loam : various manurial treatments, with slight changes in rates, have been given continuously to the plots since 1928. The manurial treatments in 1950 were as shown below, nitrogen being given in the form of nitrochalk, phosphorus as superphosphate, and potash as potassium sulphate, and the ratios expressed on a cwt.-per-acre basis.

N ..	.. 3
NP ..	.. 3:4
NK ..	.. 3:1
PK ..	.. 4:1
NPK ..	.. 3:4:1
FYM ..	.. 20 tons

All treatments were repeated in 1951 and 1952 except FYM, which was given only in 1950. Each treatment was applied to 3 sub-plots of 120 plants each plus 2 buffer rows, plots being randomised over an area of one-third of an acre. Of each 120 plants, two rows of 6 in a similar position on each plot were reserved in 1951 for the experiments on assessment of quality, the rest of the plot was used to study the effect of treatments on growth, fruit yield, visual deficiency symptoms and leaf nutrient status. These results, together with the effect of treatments on soil fertility, will be described elsewhere by Bould and Catlow. Fruit was picked when the berries were fully ripe and no unripe or damaged fruit was included in these experimental samples.

In the 1952 season, the same 12 plants were reserved, with the exception of one plot receiving NP treatment, where so many plants had died by the end of 1951 that it was considered advisable to use 12 plants at the opposite end of this plot. Also one plant in the experimental rows of an NK plot and two plants in one of the NPK plots were missing in 1952, but the necessary allowance was made for this in the statistical analysis of crop weights.

Crop Weight recording. The total weight of sound, fully-ripe berries from the experimental plants in each of the 18 plots was recorded for each pick ; any extremely large or small berries were then removed. No further grading was possible owing to the small yields from some plots, so that differences between large, medium and small fruits were not recorded.

Methods.

Tasting Panel Test.

A number of experienced tasters were selected for their ability to detect small differences in a test set of known samples of fruit ; they were also selected as being without any marked personal preferences for such components as acid and sugar. Nine tasters formed the panel in 1951, and ten in 1952. Marks were given for full, typical strawberry flavour, for texture, colour and appearance, and for acid-sugar balance, using the marking scheme adopted in 1950 by Crang, Kendall and Sturdy (1). These scores were then aggregated after suitable weighting, giving for instance more weight to flavour than to colour or acid-sugar balance, which were more variable within the sample and therefore less easy to assess fairly.

The tasting tests were made on samples of approximately 1 lb. from each of the 18 plots, divided into 3 groups of 6 at random, and labelled with coded numbers. Tasters were asked to mark one group at a time with an interval of at least 1 hour between groups, as this was found to be the maximum number which could be fairly compared at one session. The aggregate scores were analysed statistically and effects of harvest period and manurial treatment determined.

Chemical Analysis.

Sampling. The berries in a 400 g. sample of fruit from each plot were hulled and quartered, giving four 100 g. samples, which were used for the investigations described below, the exact weight of the sample being determined when necessary.

Ascorbic acid. A preliminary comparison of methods was carried out on an earlier variety of strawberry, titrating a 2% metaphosphoric acid extract of the fruit potentiometrically with phenol-indo-2:6-di-chlorophenol, and titrating a similar extract visually with potassium iodate (2). The results were found to agree within the sampling error, and the latter method was therefore adopted, as the starch-iodine end point was obtained easily and rapidly. All ascorbic acid determinations were carried out on the day of harvesting. The quarters were cut directly into a tared beaker containing 2% metaphosphoric acid, the fruit was weighed (± 0·1 g.), and the mixture blended in a Waring Blendor. Aliquots of the extract for titration were taken after straining through organdie.

Results were expressed as mg. ascorbic acid per 100 g. fresh fruit.

pH. A second quarter of the berries was used for pH determination. The samples were thoroughly crushed and mixed in a small mortar, transferred to beakers, allowed to stand for ½ hour, and the pH of the pulp was then determined with a glass electrode.

Titratable acidity. The titratable acidity was usually determined on the day of harvesting, using a hot water extract of a third quarter of the same 400 g. samples; and titrating as described by Burroughs for fruit juice and cider (3). For fruit stored before testing, two 100 g. samples of whole berries were hulled, weighed to the nearest 0·1 g., enclosed in sealed cellophane bags and deep-frozen at –23° C. until required for analysis; the whole of a weighed sample was then thawed for use, to avoid errors due to gain or loss of moisture.

The results were calculated as milliequivalents of acid per 100 g. fresh fruit.

Total sugars. Sugars were determined in either fresh or deep-frozen samples using the same hot-water extracts of 100 g. samples as for the titratable acidity determinations. Aliquots of these extracts were gently boiled for 15 minutes with 10% citric acid to complete the inversion of sucrose; they were then cooled, neutralised to litmus, cleared by the addition of neutral lead acetate and, after centrifugation, reducing sugars were determined by the method of Lane and Eynon (4).

The results were expressed as the percentage of total sugar calculated as invert per 100 g. of fresh fruit.

Pectin. 100 g. samples stored at –23° C. as described were thawed by blending with an equal volume of warm distilled water in a Waring Blendor, washed into beakers with distilled water, and the finely-divided pulp was extracted thrice with distilled water by gentle boiling for half hour periods, and finally the volume of combined filtrates was adjusted to 500 ml. The pectin in aliquots of these extracts was precipitated by the addition of sufficient acetone to bring the concentration to 60%, the acetone being added slowly with stirring. The pectin precipitate was filtered off using Whatman No. 54 papers and keeping funnels and beakers covered during the process. The precipitate was washed several times with 60% acetone, then redissolved in boiling distilled water, filtering through the same paper. The solution, after cooling and adjustment to a suitable volume, was treated by the method of Carré and Haynes to determine the pectin as calcium pectate (5).

Statistical treatment.

Analysis of variance using the *t* test for significance was applied to find the significance of the effects of time of harvesting, manurial treatment applied, and the interaction of both these factors. In 1952 the mean value of H-ion concentration was calculated for samples from each treatment, but as the difference between extreme values corresponded to less than 0·1 pH unit, no further statistical analysis was carried out upon these figures.

Results.

Effects of Manurial Treatments.

In both seasons the plots receiving N and NP (i.e. those without K) gave

TABLE I.

1951. EFFECT OF MANURIAL TREATMENTS.
(Means of 18 values ; 3 blocks × 6 harvests).

	N	NP	NK	PK	NPK	FYM	Mean	S.D.			Differences
								P=5%	P=1%	P=0·1%	* P=5% ** P=1% *** P=0·1%
Ascorbic Acid (mg. per 100 g.)	43·0	42·1	50·0	46·4	49·5	50·1	46·9	1·85	2·63	3·80	*** FYM, NK, NPK > N, NP *** PK > NP ** FYM, NK, NPK > PK > N, NP
Total Sugars (as invert) (g. per 100 g.)	5·45	4·70	5·70	5·45	5·72	5·70	5·45	0·34	0·49	0·71	*** NPK, FYM, NK, PK, N > NP
Titratable Acidity (m.e. per 100 g.)	11·19	10·21	15·42	14·66	15·08	15·36	13·64	0·909	1·292	1·870	*** NK, FYM, NPK, PK > N, NP * N > NP
Pectin as Calcium Pectate (g. per 100 g.)	0·5493	0·5303	0·5695	0·5591	0·5696	0·5810	0·5598	0·0270	0·0384	0·0556	** FYM, NPK, NK > NP * FYM > N ; PK > NP
Crop Weight (ozs. per 12 plants)	39·16	35·88	46·33	42·61	48·88	53·06	44·32	8·92	12·68	18·36	** FYM, NPK > NP * FYM, NPK > N FYM > PK. NK > NP
Tasting Test† (maximum =100)	72·7	66·1	76·8	77·5	76·5	75·1	74·1	2·96	4·13	5·98	*** PK, NK, NPK, FYM, N > NP ** PK > N * NK, NPK > N

†Means of 15 values ; 3 blocks × 5 harvests. Insufficient number of observers for Harvest 1.

TABLE II.

1952. EFFECTS OF MANURIAL TREATMENTS.

(Means of 18 values ; 3 blocks × 6 harvests).

	N	NP	NK	PK	NPK	FYM	Mean	S.D.			Differences
								P=5%	P=1%	P=0·1%	* P=5% ** P=1% *** P=0·1%
Ascorbic Acid (mg. per 100 g.)	41·2	41·0	47·2	47·6	47·0	46·2	45·02	1·70	2·41	3·51	*** PK, NK, NPK, FYM > N, NP
Total Sugars (as invert) (g. per 100 g.)	4·85	4·67	5·21	5·42	5·14	5·21	5·08	0·35	0·49	0·72	*** PK > NP ** NK, FYM > NP ** PK > N * NPK > NP * NK, FYM > N
Titratable Acidity (m.e. per 100 g.)	9·5	9·5	13·0	13·2	12·6	11·6	11·57	0·88	1·25	1·81	*** PK > N, NP ** NK, NPK, FYM > N, NP ** PK > FYM
Pectin as Calcium Pectate (g. per 100 g.)	0·417	0·423	0·461	0·454	0·467	0·448	0·445	0·040	0·056	0·082	* NPK > NP, N
Crop Weight (ozs. per 12 plants)	55·66	46·88	63·38	49·56	59·83	63·66	56·50	9·66	13·74	19·86	** FYM, NK > PK, NP * NPK > PK, NP
Tasting Test (maximum = 100)	71·96	70·74	75·93	76·57	76·79	76·71	74·76	2·14	3·04	4·40	*** NPK, FYM, PK > N, NP ** NK > N, NP

the lowest ascorbic acid figures, lowest titratable acidities, and the lowest flavour scores ; the flavour score for NP was only significantly below that for N in the 1951 season. (Tables I and II)

Total sugar was lowest in plots receiving NP ; all other treatments gave higher sugar values in both seasons ; in 1951 plots receiving N only were not significantly different from those receiving K, but in 1952 N only gave lower values than PK, NK or FYM, thus showing a tendency to sugar deficiency where K is omitted.

In 1951 the pectin content of fruit from plots receiving FYM, NPK, NK and PK was higher than that from plots receiving NP. Treatment with N gave an intermediate value which could only be differentiated statistically from the very high value given by FYM treatment, but which showed the tendency of treatments omitting K to give low values. In 1952 the pectin levels were lower but more uniform ; NP and N again gave the lowest values, differing statistically from NPK which gave the highest values of the series for this season.

The best crop weights in 1951 were from FYM, NPK and NK plots in that order, with N and NP lowest of the series, while in 1952 the order of plots giving highest yields was FYM, NK, NPK, all significantly greater than PK and NP : but the yield from plots receiving N only was near the average and not to be differentiated from other values.

Thus the omission of K has in general resulted in a lowering of both yield and quality, both effects being accentuated by addition of P. It was the opinion of the tasting panel that fruit from the NP plots was always lacking in full flavour and aroma, also poor in colour, and could almost invariably be picked out from the coded samples by these characteristics. Fruit from plots receiving N only was similar in type but not so consistently inferior throughout both seasons.

Effects of Harvest.

In 1951 the highest crop weights were obtained in the 4th, 5th and 2nd harvests, in that order (Table III) : the eating quality was best in the 2nd harvest, with slight deterioration in the 5th and 6th picks. In 1952 (Table IV), both eating quality and yield were at a maximum in the 3rd harvest. In both years the first pick gave insufficient fruit for both tasting test and analysis, and the latter was given priority.

Total sugar and titratable acidity in 1952 were highest in the first half of the season, particularly in the 2nd harvest, while in 1951 the acidity showed no significant variation throughout the season, and sugar was highest in the 5th and 6th picks. Ascorbic acid at the beginning and end of the 1951 season was relatively high, but in 1952 reached a maximum value in the heavy 3rd pick, the values being lowest in the 5th and 6th harvests.

Pectin was unusually high in the small 1st harvest of 1951, slightly lower in harvests 4, 2 and 3, in that order, and the lowest in the 5th and 6th picks. In 1952 the highest pectin was given by the 3rd harvest, and was again lowest in the 5th and 6th picks.

In connection with these effects of harvesting periods, it is of interest to note that in 1951 the weather immediately before and during practically the whole of the picking season was fine and sunny with normal temperatures,

TABLE III.

1951. EFFECTS OF HARVEST.

(Means of 18 values ; 3 blocks × 6 treatments).

	H_1	H_2	H_3	H_4	H_5	H_6	Mean	S.D.			Differences
								P=5%	P=1%	P=0·1%	* P=5% ** P=1% *** P=0·1%
Ascorbic Acid (mg. per 100 g.)	53·2	46·8	42·3	42·0	47·5	49·0	46·9	1·63	1·94	2·16	*** $H_1 > H_6$, H_5, $H_2 > H_3$, H_4 $H_6 > H_2$
Total Sugars (as invert) (g. per 100 g.)	5·29	5·02	5·20	5·15	5·90	6·16	5·45	0·28	0·34	0·37	*** H_6, $H_5 > H_1$, H_3, H_4, H_2
Titratable Acidity (m.e. per 100 g.)	13·72	13·53	13·24	13·89	14·48	12·98	13·64	2·03	2·42	2·70	not significant
Pectin as Calcium Pectate (g. per 100 g.)	0·6355	0·5828	0·5413	0·5910	0·5257	0·4839	0·5598	0·0243	0·0291	0·0324	*** $H_1 > H_4$, $H_2 > H_3 > H_6$ $H_5 > H_6$
Crop Weight (ozs. per 12 plants)	20·67	55·00	27·22	69·45	55·83	37·78	44·32	6·34	9·02	13·06	*** $H_4 > H_5$, $H_2 > H_6 > H_3$, H_1 * $H_3 > H_1$
Tasting Test (maximum=100)	—	77·0	74·0	74·2	72·6	72·6	74·1	2·2	2·9	3·8	*** $H_2 > H_5$, H_6 ** $H_2 > H_3$ * $H_2 > H_4$

TABLE IV.

1952. EFFECTS OF HARVEST.

(Means of 18 values ; 3 blocks × 6 treatments).

	H_1	H_2	H_3	H_4	H_5	H_6	Mean	S.D.			Differences
								P=5%	P=1%	P=0·1%	* P=5% ** P=1% *** P=0·1%
Ascorbic Acid (mg. per 100 g.)	49·0	50·2	50·9	43·3	39·3	37·6	45·05	1·54	2·04	2·65	* $H_3 > H_1$ *** H_3, H_2, $H_1 > H_4$, H_5 ** $H_4 > H_5 > H_6$
Total Sugars (as invert) (g. per 100 g.)	5·17	5·80	5·32	4·68	4·75	4·79	5·09	0·17	0·22	0·29	*** $H_2 > H_3$, $H_1 > H_6$, H_5, H_4
Titratable Acidity (m.e. per 100 g.)	12·6	12·8	11·3	12·0	10·8	11·6	11·85	0·57	0·75	0·98	*** H_2, $H_1 > H_6$ *** $H_4 > H_5$ * $H_2 > H_4 > H_3$
Pectin as Calcium Pectate (g. per 100 g.)	0·460	0·471	0·495	0·463	0·401	0·381	0·445	0·047	0·062	0·082	*** H_3, $H_2 > H_6$; $H_3 > H_5$ ** $H_2 > H_5$; H_4, $H_1 > H_6$
Crop Weight (ozs. per 12 plants)	19·38	44·55	142·50	62·66	50·05	19·50	56·44	9·56	12·66	16·46	*** $H_3 > H_4$, $H_5 > H_6$, H_1 ; *** H_3, $H_4 > H_2$ * $H_4 > H_5$
Tasting Test (maximum=100)	—	76·75	79·60	73·00	72·10	72·40	74·76	1·78	2·39	3·13	*** H_3, $H_2 > H_4$, H_6, H_5 ** $H_3 > H_2$

with the exception of a few thundery showers. In 1952 the picking season was preceded by 3 days of cool, dull weather and a little rain. Between harvests 1 and 3 the temperature rose to normal and there was a considerable amount of sunshine with only traces of rain. After the 3rd harvest, rainfall and sunshine became variable, but the temperature remained consistently high and normal for the time of year. These factors probably account very largely for the more even crop weights in 1951 as compared with the extremely heavy yield in the 3rd harvest of 1952. It may also be remarked that in spite of the great increase in weight of fruit in this 3rd crop, there was no corresponding drop in either ascorbic acid or other constituents analysed, showing that there was not a dilution effect due to excess uptake of water.

If the mean values of the various fruit components for each treatment over the whole of the 1952 season are compared with the values for 1951, it will be seen that differences were not great, but the general tendency was for the quantities of soluble constituents in 1952 to be lower than in 1951 whereas the crop weight for 1952 was higher. The quality ratings in the two seasons were however very similar. The plants receiving FYM, NPK and NK treatments were in full vigour at the end of both seasons ; those receiving PK were fairly vigorous, while plants not receiving potash showed characteristic signs of K deficiency, particularly on NP plots, where many plants had died out by the end of 1951.

Interaction of harvest and manurial treatment effects.

Tables V—IX show that whereas in 1951 the tasting quality scores were the only ones to show significant differences due to the varying effect of manurial treatments with the time of harvest, in 1952 all the components except pectin were affected.

In 1951 the deleterious effect of NP treatment upon the tasting quality scores was apparent from the second harvest ; further differences between treatments were obvious in the later harvests where FYM, NPK and NK showed increasing superiority over N and NP treatments, with PK remaining as a treatment of intermediate value (Table V). In the 1952 season, similar effects occurred with tasting scores (Table VI), with the increase in proportionate differences between manurial treatments again becoming greater after the third harvest. In the case of ascorbic acid and titratable acidity, significant differences were apparent at all harvests between plots receiving FYM, NPK, NK and PK, and those receiving N and NP (Tables VII and VIII). The sugar content also showed differences at all harvests (Table IX) ; NP treatment always gave the lowest values : the next-lowest were from plots receiving N only.

Summary.

Auchincruive Climax strawberries were harvested in ripe condition during the 1951 and 1952 seasons, from plots which had received the same manurial treatment since 1928. The levels of N, P and K had been varied and an FYM treatment included for comparison. The plants were in their 1st and 2nd years of cropping.

Quality was assessed by a tasting test score, and by determination of ascorbic acid, total sugars, titratable acidity and pectin levels ; crop weights were also recorded.

TABLE V.

1951. TASTING TEST SCORES.

INTERACTION OF HARVEST UPON MANURIAL TREATMENT EFFECT.

(Means of 3 values for blocks ; maximum score 100).

Harvest	Treatment						Mean	Differences *** P = 0·1% ** P = 1·0% * P = 5·0%
	N	NP	NK	PK	NPK	FYM		
2. ..	76·0	72·5	77·0	78·8	78·5	78·5	77·0	* PK, NPK, FYM > NP
3. ..	76·5	65·2	76·5	74·5	75·9	74·7	74·0	*** N, NK > NP ** NPK, FYM, PK > NP
4. ..	73·0	64·1	79·7	78·7	77·5	73·5	74·2	*** NK, PK, NPK > NP ** FYM, N > NP
5. ..	66·9	69·4	76·9	75·6	71·4	75·2	72·6	*** NK > N ** PK, FYM > N ; NK > NP * NK > NPK
6. ..	70·7	59·1	73·3	80·2	78·7	73·4	72·6	*** PK, NPK, FYM, NK, N > NP ; PK > N ** NPK > N * PK > FYM, NK ; NPK > NK
Mean..	72·7	66·1	76·8	77·5	76·5	75·1		

S.D. for P = 5% 5·4
P = 1% 7·1
P = 0·1% 9·4

TABLE VI.

1952. TASTING TEST SCORES
(maximum score=100).
INTERACTION OF HARVEST UPON MANURIAL TREATMENT EFFECT.
(Means of 3 values for blocks).

Harvest	Treatment						Means	S.D.			Differences
	N	NP	NK	PK	NPK	FYM		P=5%	P=1%	P=0·1%	* P=5% ** P=1% *** P=0·1%
2.	75·0	75·4	77·5	76·4	78·8	77·5	76·8	4·36	5·84	7·65	not significant
3.	79·1	76·6	77·5	82·8	80·8	81·0	79·6				** PK > NP * PK > NK
4.	71·9	68·8	74·1	75·1	73·8	74·1	73·0				* PK, NK, FYM, NPK > NP (N=NP)
5.	65·1	67·2	77·0	73·5	75·2	74·9	72·1				*** NK, NPK > NP, N *** PK > N ** FYM > N, NP; PK > NP
6.	68·8	65·7	73·2	75·2	75·3	76·1	72·4				*** FYM > NP ** NPK, PK > N, NP ** FYM > N; NK > P
Means ..	72·0	70·7	75·9	76·6	76·8	76·7					

TABLE VII.

1952. ASCORBIC ACID
(mg. per 100 g.).
INTERACTION OF HARVEST UPON MANURIAL TREATMENT EFFECT.
(Means of 3 values for blocks).

Harvest	Treatment						Mean	S.D.			Differences
	N	NP	NK	PK	NPK	FYM		P=5%	P=1%	P=0·1%	* P=5% ** P=1% *** P=0·1%
1.	44·4	43·7	52·5	49·9	52·7	50·4	49·0	2·57	3·40	4·42	*** NPK, NK, FYM, PK > N, NP * NPK > PK
2.	44·2	46·9	53·5	51·0	52·1	53·1	50·2				*** NK, FYM, NPK > NP, N *** PK > N ** PK > NP
3.	48·1	45·6	52·8	55·1	52·6	51·2	50·9				*** PK, NK, NPK > N, NP *** FYM > NP ** PK > FYM * FYM > N
4.	39·8	39·1	45·0	45·5	45·1	45·3	43·3				*** PK, FYM, NPK, NK > N, NP
5.	35·5	35·5	41·6	42·4	41·3	39·1	39·3				*** PK, NK, NPK > N, NP ** FYM > N, NP * PK > FYM
6.	35·1	34·9	38·0	41·4	38·0	37·9	37·6				*** PK > N, NP ** PK > FYM * NK, NPK, FYM > N, NP
Means ..	41·2	41·0	47·2	47·6	47·0	46·2					

TABLE VIII.

1952. TITRATABLE ACIDITY
(m.e. per 100 g.).
INTERACTION OF HARVEST UPON MANURIAL TREATMENT EFFECT.
(Means of 3 values for blocks).

Harvest	Treatment						Means	S.D.			Differences
	N	NP	NK	PK	NPK	FYM		P=5%	P=1%	P=0·1%	* P=5% ** P=1% *** P=0·1%
1.	10·4	11·1	13·9	12·6	13·4	14·4	12·6	1·40	1·85	2·41	*** FYM, NK > NP, N ; NPK > N ** NPK > NP ; PK > N * FYM > PK > NP
2.	10·3	9·6	14·5	14·2	14·3	14·4	12·8				*** NK, FYM, PK, NPK > N, NP
3.	9·4	8·9	12·3	12·7	11·8	12·8	11·3				*** PK, FYM, NK > N, NP *** NPK > NP ** NPK > N
4.	9·5	9·9	12·1	13·3	13·1	14·1	12·0				*** FYM, PK, NPK > NP, N *** NK > N ** NK > NP
5.	8·5	8·5	11·7	12·5	11·7	11·7	10·8				*** PK, NK, NPK, FYM > N, NP
6.	8·8	8·6	13·2	13·7	12·5	12·9	11·6				*** PK, NK, FYM, NPK > N, NP
Means ..	9·5	9·5	13·0	13·2	12·6	11·6					

TABLE IX.

1952. TOTAL SUGARS

(g. per 100 g.).

INTERACTION OF HARVEST UPON MANURIAL TREATMENT EFFECT.

(Means of 3 values for blocks).

Harvest	Treatment						Means	S.D.			Differences
	N	NP	NK	PK	NPK	FYM		P=5%	P=1%	P=0·1%	* P=5% ** P=1% *** P=0·1%
1.	4·87	4·73	5·53	5·20	5·27	5·43	5·17	0·41	0·55	0·71	*** NK > NP ** NK > N * FYM, NPK, PK > NP ; FYM > N
2.	5·33	5·23	5·83	6·03	6·23	6·10	5·80				*** NPK, FYM > N, NP ; PK > NP ** PK > N * NK > N, NP
3.	5·30	4·97	5·40	5·50	5·37	5·40	5·32				* PK, FYM, NK > NP
4.	4·60	4·33	4·74	5·13	4·52	4·70	4·68				** PK > NP * PK > FYM, N, NPK ; NK > NP
5.	4·60	4·37	4·77	5·33	4·70	4·70	4·75				*** PK > NP ** PK > FYM, NPK, N * PK > NK
6.	4·40	4·37	4·97	5·33	4·73	4·93	4·79				*** PK > N, NP ** PK > NPK ; NK > N, NP * FYM > N, NP
Means ..	4·85	4·67	5·21	5·42	5·14	5·21					

In both seasons, plots receiving N and NP gave the fruit of poorest quality and lowest yield ; FYM, NPK and NK gave consistently high yields and quality in both years, while PK gave fruit intermediate in both quality and yield in 1951, but of improved quality in 1952.

In both years the fruit of best quality was obtained in the earlier half of the season ; the highest crop weights were given in the middle of the harvesting period. The results of manurial treatments became increasingly apparent at the later harvests in each year.

The results of the tests for the two seasons therefore confirm the earlier findings of Knight and Wallace (6), that an adequate supply of potash is of major importance in the nutrition of strawberries, and show that the application of P to K deficient plants enhances the deterioration in both yield and quality of fruit.

Acknowledgments.

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A COMPARISON OF SOME VARIETIES OF FRUIT PRESERVED BY BOTTLING AND BY FREEZING.

PROGRESS REPORT III.

By ALICE CRANG and MARGARET STURDY.

A comparison of the qualities of different varieties of fruit preserved by bottling and by freezing has been continued during the 1952 season. Previously (1) (2) the fruit was also preserved by canning, but since the bottled and canned fruit results were generally similar, the latter process was omitted this season.

Unless otherwise stated, the fruit was grown at Long Ashton Research Station.

Preserving Methods.

The bottling and freezing methods were similar to those used in 1951 (2) ; most bottled fruit being processed in a water bath raised from cold to 180° F. in $1\frac{1}{2}$ hours and maintained at that temperature for 15 minutes, and the fruit and syrup for freezing being sealed in bags made from " Cellophane " grade MSAT 300/30, frozen and stored in a freezer with a temperature range from –10° to 0° F.

Thawing Method.

The frozen fruit was put overnight in a domestic refrigerator and finally thawed for 3 hours at room temperature before the packets were opened and the contents judged against the freshly opened bottled fruit. This method differs from that used previously when the frozen fruit was taken straight from the freezer for thawing.

The frozen gooseberries were cooked and cooled before they were judged, and the blackberries judged both raw and after cooking.

Judging Methods.

The fruit was judged after 4 to 6 months' storage.

At least eight of a panel of ten people judged each variety of fruit. As previously, the fruit was put in identical glass bowls and marked by a code number for judging : a maximum of 10 marks each was allowed for colour, texture and flavour. The markings given for sugar/acid balance and for size have been omitted from the results unless they were of particular interest.

Results.

Blackberries.

The varieties Ashton Cross, Himalaya Giant and Merton Early were each preserved by bottling, by freezing with sugar (9 oz. fruit to 3 oz. sugar) and by freezing with 30° Brix syrup (9 oz. fruit to 6¼ fl. oz. syrup). The frozen fruit was judged both raw and cooked. The average of 45 scores for the varieties and 27 for the processes is given in Table I.

TABLE I.

BLACKBERRIES.

Variety. Average of all Processes	Colour	Texture	Flavour
Ashton Cross	7·4	7·6	7·2
Himalaya Giant	7·8	7·5	7·3
Merton Early	7·3	7·5	6·7
Process. Average of all Varieties			
Bottled	6·8	7·5	7·5
Frozen with sugar uncooked ..	8·2	7·8	7·2
,, ,, ,, cooked	7·8	7·7	7·2
,, ,, syrup uncooked ..	7·7	7·4	6·6
,, ,, ,, cooked	7·1	7·4	7·0

The results follow very closely those obtained for these three varieties of blackberries in 1950 (1).

Varieties.

Ashton Cross. The flavour of the bottled, but not of the frozen fruit was considered slightly above the average, but this variety was not outstanding.

Himalaya Giant. The best of the series for colour and flavour though the differences were not great.

Merton Early. As previously, this variety was slightly poorer in colour and flavour than the other two.

Preserving Methods.

Freezing was preferred to bottling for colour, and the fruit frozen with sugar was the best in this respect.

There was little difference in the texture between the varieties or processes.

The bottled fruit was preferred to the frozen for flavour, but the differences were not great.

Cherries.

Only two varieties of cherries were tested ; *Circassian*, obtained from Kent, graded into ripe and under-ripe fruit and *Morello* (just ripe). We are indebted to C. E. Hudson, Esq., of Wisbech, for sending us the latter fruit.

The fruit was preserved with 30° Brix syrup to give a proportion of 3 parts fruit to one of sugar (by weight).

The results given in Table II are the average of 10 scores.

TABLE II.

CHERRIES.

Variety	Bottled			Frozen		
	Colour	Texture	Flavour	Colour	Texture	Flavour
Circassian ripe ..	6·9	7·4	7·4	8·4	8·5	7·8
,, under-ripe	6·0	7·7	7·2	7·9	8·0	6·5
Morello	6·8	7·5	7·0	9·0	7·8	5·6

Varieties.

Circassian. The ripe frozen cherries were above the average in colour and texture when tested immediately they were sufficiently thawed, and of quite a good flavour. They were only fairly good in all respects when bottled. The under-ripe fruit scored less for colour and flavour in each group. The sugar/acid balance was satisfactory.

Morello. Unusually even and bright in colour when frozen but rather poor in flavour, and very sour. The bottled fruit was of only fair colour, but better for flavour and rated between slightly and moderately sour.

Preserving Methods.

The colour of the frozen fruit was very good when judged soon after it had thawed, although it deteriorated on standing. In comparison, the colour of the bottled fruit was only fair.

The texture of the frozen fruit was this year preferred to that of the bottled fruit. This is the opposite effect to that noted last year, and may have been due to the change in method of thawing.

Citrus Fruits.

Grapefruit, Jaffa oranges and tangerines were tested for different freezing treatments, but as only one variety of each kind of fruit was preserved, the results have been considered together in Table III.

The fruit was peeled, and in one series the segments were cut free from the tissues, then frozen immediately, whereas in another the segments were dipped for 30 sec. in boiling 1% lye solution and rinsed in cold water. Half the fruit in each treatment was frozen alone and the other half in an equal weight of 30° Brix syrup. One sample of grapefruit and one of oranges was preserved by bottling.

TABLE III.

CITRUS FRUITS.

Treatment	Colour			Texture			Flavour		
	Grapefruit	Orange	Tangerine	Grapefruit	Orange	Tangerine	Grapefruit	Orange	Tangerine
Frozen :									
(a) slices cut from tissues —no syrup	8·6	8·0	7·5	7·9	7·1	6·8	6·1	7·1	6·0
(b) slices cut from tissues —in syrup	8·1	8·3	8·3	7·6	7·9	7·5	7·3	6·6	8·0
(c) lye-dipped —no syrup	8·0	8·7	8·8	7·6	7·9	8·5	5·1	5·1	5·7
(d) lye-dipped —in syrup	9·0	9·0	9·3	7·4	8·0	9·0	6·1	5·9	6·0
Bottled :									
(e) slices cut from tissues —in syrup	—	7·0	—	—	7·3	—	—	5·9	—
(f) lye-dipped —in syrup	4·6	—	—	7·1	—	—	5·4	—	—

Preserving Methods.

The colour of the fruit which had been lye-dipped prior to freezing with syrup was unusually good, and nearly all the freezing treatments were rated better than the average for colour, whereas the bottled grapefruit was poor, and the bottled orange only fairly good. There was less variation in texture, but the fruit preserved by freezing was generally preferred to the bottled fruit.

The flavour was disappointing, especially in the lye-dipped fruit. Tangerines and grapefruit were preferred when preserved in syrup, and the latter fruit would have been improved with a higher proportion of sugar for most palates, but the orange slices were preferred without sugar.

Gooseberries.

The varieties Keepsake, Leveller and Whinham's Industry were judged for the third successive year. The gooseberries were frozen without sugar, and after storage, thawed sufficiently to separate the berries, which were then cooked in syrup. The proportions used were 9 oz. fruit and 6½ fl. oz. of 40° Brix syrup for each test. The figures given in Table IV are the mean of 10 scores.

TABLE IV.

GOOSEBERRIES.

Variety	Bottled			Frozen		
	Colour	Texture	Flavour	Colour	Texture	Flavour
Keepsake	6·5	7·7	7·6	8·3	7·1	6·9
Leveller	8·2	7·3	7·8	8·2	7·1	7·5
Whinham's Industry	7·1	7·6	7·9	7·5	7·0	7·1

Varieties.

Keepsake. The pale colour of the bottled fruit was again noticeable, but this year that of the frozen fruit was judged the best of the three. The flavour of the frozen fruit was only fairly good, but a slight improvement on the marks given previously.

Leveller. This fruit again gained the highest total marks for each method of processing, and the marking is very similar to that given last year.

Whinham's Industry. This variety scored higher marks in colour, flavour and texture than in previous years when bottled, but was again marked slightly below the other two varieties when frozen.

The differences in the total scores for the varieties were not significant this year.

Preserving Methods.

The colour of the frozen fruit was better than that of the corresponding bottled fruit in two varieties and similar in the third, but this was offset by the slightly poorer texture and flavour in the frozen fruit. There was an improvement in the wholeness and texture of the frozen fruit compared with last year due probably to the method employed of thawing sufficiently to separate the berries before these were cooked in syrup.

The proportions of fruit to syrup used were considered satisfactory.

Plums.

Thirteen varieties of plums, damsons or gages were compared after preserving by bottling and freezing in the proportions of approx. 2 parts of fruit to 1 part of 30° Brix syrup. The frozen fruit was thawed but not cooked. Since the preserving treatment so altered the appearance and flavour of the fruit, the two treatments of each variety were coded, but placed alongside of one another for judging.

Each figure in Table V represents an average of 9 scores.

Varieties.

Apricot Gage. This fruit was judged to be outstandingly good when preserved by freezing but only of average quality when bottled.

TABLE V.

PLUMS AND GAGES.

Variety	Bottled			Frozen		
	Colour	Texture	Flavour	Colour	Texture	Flavour
Apricot Gage	7·0	8·6	7·2	9·9	8·8	8·9
Belle de Louvain	5·4	7·2	7·2	9·0	7·0	8·4
Count Althann's Gage	7·4	7·1	7·8	8·8	6·8	8·2
Early Transparent Gage ..	6·9	7·6	7·6	7·3	7·3	8·2
Frome Cross	6·8	7·0	7·2	8·9	6·2	7·0
Jefferson	8·3	6·9	7·6	8·2	7·0	7·9
Laxton's Bountiful	6·0	7·0	5·0	8·3	6·6	3·9
Marjorie's Seedling	4·7	6·7	7·1	8·0	6·9	7·8
Pershore (Yellow Egg)	8·3	8·0	7·3	7·3	7·8	8·0
Pond's Seedling	7·1	7·4	7·4	9·3	7·1	8·0
Shropshire Prune Damson ..	7·0	8·1	7·7	8·7	6·7	7·5
Victoria	7·0	7·7	5·7	9·4	7·4	7·0
Long Ashton Seedling NA.10 ..	8·1	7·1	7·0	8·4	7·0	8·6
Mean score	6·9	7·4	7·1	8·6	7·1	7·7

In previous tests (1) it gained the highest total score of the bottled varieties, but was not then frozen.

Belle de Louvain. This variety was judged to be more suitable for freezing than bottling. The colour and flavour of the frozen fruit were very good, whereas they were below the average when bottled. It was noted previously to be one of the least satisfactory plums tested for bottling.

Count Althann's Gage. The colour and flavour of the frozen fruit were both very good, but the skins were tough, so losing marks on texture. The quality of the bottled fruit was quite good.

Early Transparent Gage. The frozen fruit was considered a little better than the bottled fruit, but the differences were small. For colour it was fairly good, and the flavour was above the mean score for both processing methods.

Frome Cross. This fruit has a damson colour which was well retained in the frozen product, but in comparison, the bottled fruit colour was only fairly good. The flavour was fairly good in both, but not rated so highly as in previous tests on the heat-processed fruit. The tough texture spoils the uncooked fruit.

Jefferson. The colour and flavour were satisfactory in both methods of preserving, but the skins were a little tough.

Laxton's Bountiful. The flavour was considered just passable in the bottled, and very poor, with a foreign taint, in the frozen fruit. The colour was fair in the bottled and quite good in the frozen fruit.

Marjorie's Seedling. The colour was quite good in the frozen but unattractive in the bottled fruit, and the skins were tough. The flavour was fairly good in both.

Pershore (*Yellow Egg*). One of the best of the series for bottling and quite good for freezing, though the colour was not retained so well in the latter method.

Pond's Seedling. Unusually good for colour and quite good for flavour when preserved by freezing, and fairly good in colour, texture and flavour when bottled, though its size makes it a difficult fruit to pack into containers.

Shropshire Prune Damson. A good fruit for bottling or freezing, but the frozen fruit needs cooking before serving, otherwise it is tough.

Victoria. The colour was unusually good when frozen and quite good when bottled, but the flavour was again disappointing in the bottled fruit.

Long Ashton Seedling N.A.10. This new variety, a cross between Coe's Golden Drop and Giant Prune, retained its yellow colour well in the bottled and frozen fruit. The flavour was fairly good in the bottled and good in the frozen fruit.

Processing Methods.

The frozen fruit was generally very attractive in colour when first thawed, but it deteriorated quickly on standing, as might be expected ; the fresh fruit flavour was also well retained in most of the varieties. This fruit was not cooked after freezing, but only in the damsons was the texture marked appreciably below that of the bottled fruit.

Pershore (Yellow Egg) was the only variety in which the colour was better in the bottled than in the frozen fruit. The flavour of the kernel was very noticeable in the bottled fruit and frequently masked the fresh fruit flavour entirely, but this was liked by some of the judges.

Strawberries.

Three varieties of strawberries were preserved by freezing the whole and sliced fruit with both sugar and sugar syrup and by bottling the whole fruit in syrup. The proportions of fruit to sugar used was 3:1. One variety was preserved without sugar and sweetened on thawing. The marks awarded the frozen fruit were similar in each of the four processes, and the average is given in Table VI. The Cambridge Early and Royal Sovereign strawberries were obtained from Cheddar, picked and processed within a few hours. Ponceau 2R dye (·08%) was added to the syrup used in the bottled fruit.

Varieties.

Auchincruive Climax. The colour of the frozen fruit was outstandingly good but the marks for flavour were very variable, to bring the average to fairly good.

Cambridge Early. The frozen fruit colour and texture were good and the flavour average, but the bottled fruit was the poorest of the three. In spite of some dye used in the syrup, colour was unacceptable.

Royal Sovereign. The bottled fruit was rated of fair quality, the fruit responding better to the dye than the other two varieties, but the colour when frozen was the poorest of the three.

TABLE VI.

STRAWBERRIES.

Variety	Bottled			Frozen		
	Colour	Texture	Flavour	Colour	Texture	Flavour
Auchincruive Climax ..	5·9	7·6	7·3	9·1	7·6	7·2
Cambridge Early ..	4·7	7·0	6·0	8·1	7·9	7·5
Royal Sovereign ..	6·3	7·0	6·6	7·0	7·8	7·5
Auchincruive Climax .. (sweetened on thawing)	—	—	—	7·4	6·8	5·2

Processing Methods.

The outstanding difference between the frozen and bottled fruit was due to the colour. Although dye is necessary in the bottled fruit to make an acceptable product, the result is a uniform colour which looks artificial when compared with the natural variation in tints which are retained so well in the frozen fruit. The texture and flavour of the frozen fruit were generally preferred to those of the bottled fruit.

The variations due to adding sugar or sugar syrup to the whole and to the sliced fruit of each variety before freezing were small, but the fruit frozen without sugar and sweetened on thawing was inferior to that frozen with sugar.

Discussion.

The comparisons in the 1952 season were restricted to fruit preserved by bottling and by freezing. The eight varieties of soft fruit that were also tested in 1950 and 1951 have shown similar results, but seasonal differences have been noted in the gooseberries. Cherries, citrus fruits, plums and one variety of strawberries have been frozen for the first time in these trials. The altered method of thawing frozen fruit has shown an improvement in texture, compared with previous seasons.

The importance of the variety was especially marked in the tests on plums : with most varieties the frozen fruit was preferred to the bottled fruit, but with *Pershore* (*Yellow Egg*) the order was reversed. Although the fruit when first thawed was generally preferred to the bottled fruit, the quality of the former deteriorated rapidly after standing for more than ½ hour.

It is difficult to compare these trials with those reported by Campden Research Station (3), (4) in 1950 and 1951, as different varieties were used, but it is interesting to note that both in canning and freezing *Royal Sovereign* strawberries have been rated poor, and the opinion is expressed that modern strains of this strawberry have lost the full flavour of 15–20 years ago.

Summary.

The results of tasting tests on 27 varieties of fruit preserved by bottling and by freezing are given. The frozen fruit was again found to be generally

superior in colour to the heat-processed fruit, but the flavour varies considerably and no generalization can be made. A different method of thawing from that used in previous trials has given improved texture with most frozen fruit.

Acknowledgments.

Our thanks are due to Miss M. Hooper for assistance in the processing and judging and to all the members of the tasting panel.

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A COMPARISON OF SOME VARIETIES OF VEGETABLES PRESERVED BY CANNING AND BY FREEZING.

PROGRESS REPORT II.

By ALICE CRANG and MARGARET STURDY

A comparison has been made of the colour, texture and flavour of varieties of vegetables preserved by canning and by freezing under domestic conditions during the 1952 season. Since the bottled and canned vegetables were generally similar in previous tests, the bottled vegetables were omitted in these trials.

Vegetables Used. Unless otherwise stated the vegetables were grown at Long Ashton Research Station. As previously, they were preserved as soon as possible after picking.

Canning Methods. The vegetables were preserved in A2½ S.R. cans, using the recommended proportions of vegetable to brine, processed at 10 lb. steam pressure for the required time,* and stored at room temperature.

Freezing Methods. All the vegetables, except the mushrooms, were scalded in boiling water, cooled and packed (without brine) as in the previous trials (1). The treatment given to the mushrooms is noted under that section.

Judging Methods. The frozen vegetables were cooked, and the canned ones re-heated just before they were judged. The same method of scoring was used as previously, but in this report the average scores for colour, texture and flavour (each marked out of a maximum of 10) are given separately. The scores given are the averages of the assessments of at least 9 of the 10 people on the testing panel.

*See Ministry of Agriculture Bulletin 21, 7th edition, for processing details.

Results.

Beans (Broad).

The beans were tasted after 4 months' storage.

TABLE I.

BEANS (BROAD).

Variety	Canned			Frozen		
	Colour	Texture	Flavour	Colour	Texture	Flavour
Harlington	4·4	7·7	5·9	8·3	8·2	7·4
Sussex Wonder	4·3	7·1	6·6	9·1	6·3	7·7
Taylor's Windsor White ..	4·7	7·6	6·1	8·6	8·1	7·4

Processes.

The three varieties all proved unacceptable in colour when canned, due to the skins turning a dark brown, and only fair in flavour, but it should be noted that these varieties are not usually recommended as suitable for canning.

The same varieties were rated good or very good in colour and quite good in flavour when preserved by freezing.

There was no significant difference between the varieties.

Beans (Dwarf or Runner).

The varieties Masterpiece and Princeps were preserved for the second season in these trials, and the remaining varieties for the first time. The smaller beans were preserved whole and the larger varieties were sliced for preserving. They were judged after 3½–4 months' storage. Pea green colour (0·01%) was added to the brine of all the canned beans except Golden Wax.

TABLE II.

BEANS (DWARF AND RUNNER).

Variety	Canned			Frozen		
	Colour	Texture	Flavour	Colour	Texture	Flavour
Comtesse de Chambord (Whole)	6·9	5·6	5·6	7·8	6·2	7·2
Golden Wax (Whole)	3·7	7·6	6·1	6·6	7·2	7·1
Kelvedon Wonder (Sliced) ..	5·9	7·4	6·9	9·7	7·4	7·2
Masterpiece (Whole)	6·3	7·6	5·7	8·0	8·4	8·0
,, (Sliced)	—	—	—	9·1	8·7	7·3
Princeps (Sliced)	4·8	5·1	6·3	7·7	4·8	7·0

Varieties.

Comtesse de Chambord. The colour was fairly good but the texture of this variety was rather poor in both the canned and frozen samples.

Golden Wax. The colour was very unattractive in the canned and the poorest of the series in the frozen beans.

Kelvedon Wonder. The frozen sample was of excellent colour, and quite good texture and flavour, but the canned sample was only fair in colour.

Masterpiece. Both methods of freezing this bean gave good results, the sliced beans being better in colour but not quite so good in flavour as the whole ones. In comparison the colour and flavour of the canned sample was only fair.

Princeps. The colour and texture of both canned and frozen beans was poorer in 1952 than in the 1951 tests, suggesting that they may have been rather too mature this year when picked.

Processing.

The colour of the frozen beans was much more attractive than that of the canned ones, in spite of the dye used to improve the appearance of the latter. The differences in texture were slight. The flavour of the frozen beans was also preferred to that of the canned ones for each variety.

Carrots.

Two varieties of carrots were preserved by canning and by freezing the small whole carrots, and by freezing the sliced ones. They were stored for 3½ months before tasting. The results are given below.

TABLE III.

CARROTS.

Variety	Canned			Frozen		
	Colour	Texture	Flavour	Colour	Texture	Flavour
Early Nantes (Whole) ..	9·1	7·4	6·8	9·4	8·1	8·3
,, ,, (Sliced) ..	—	—	—	9·2	8·3	8·0
James's Scarlet (Whole) ..	8·4	8·1	7·6	8·9	8·4	7·9
,, ,, (Sliced) ..	—	—	—	9·0	8·0	8·2

Processes.

The colour of all the carrots was very good, there being a slight preference for the frozen ones. The flavour of the frozen carrots was quite satisfactory and the canned fairly good. There was little difference in the quality of the whole or sliced frozen carrots.

Mushrooms.

Good quality cultivated mushrooms were obtained from Wrington. They were divided so that an equal number of small and larger mushrooms were used in each treatment and those of from 1 to 3 inches in diameter were cut into halves, quarters or eighths according to size. The heat-processed mushrooms were preserved in 12 oz. glass bottles, processed for 30 min. at 10 lb. steam pressure. The following treatments were given :—

	Pre-treatment	*Preservation method*	*Final cooking method*
(i)	Scald 4 min., cool in iced water	Bottled with liquor from scalding water	Re-heat with pinch of salt.
(ii)	,,	Frozen	Stewed in $\frac{1}{4}$-pint salted water.
(iii)	,,	,,	Sauté in 1 tablesp. lard.
(iv)	Sauté in 1 tablesp. lard for 12 min.	Bottled	Re-heated.
(v)	,,	Frozen	,,
(vi)	No treatment	Frozen	Stewed in $\frac{1}{4}$-pint salted water.
(vii)	,, ,,	,,	Sauté in 1 tablesp. lard.

The tasting results, judged after 8 months' storage, are given in Table IV, in which the numbers in the first column refer to the methods listed above.

TABLE IV.

MUSHROOMS.

Preserving and final cooking method	Pre-treatment								
	Scald			Sauté			No treatment		
	Colour	Texture	Flavour	Colour	Texture	Flavour	Colour	Texture	Flavour
Bottled and re-heated (i) and (iv) . .	6·6	7·8	6·1	7·6	7·9	7·5	—	—	—
Frozen and stewed (ii) and (vi) . .	5·8	3·8	6·4	—	—	—	7·0	6·3	7·3
Frozen and sauté (iii) and (vii) . .	6·0	3·5	5·0	—	—	—	8·0	6·5	8·6
Frozen and re-heated (v)	—	—	—	7·8	6·8	7·4	—	—	—

It will be seen that the texture of the mushrooms that had been scalded before freezing was very tough (as found previously), but was fairly good in the samples frozen without preliminary treatment and in those sautéd before freezing. The bottled mushrooms were slightly better in texture. The flavour of the mushrooms sautéd after freezing was the only one considered above the average.

Peas.

Four of the five varieties of peas tested had been compared in 1951. Pea green dye (0·01%) was added to the brine of the canned samples. All were stored for 5 months. The results are assembled in Table V.

Varieties.

The differences in the quality of the varieties were not marked.

Dwarf Champion, tested for the first time in these trials, was of very good colour and quite good texture and flavour when frozen, but of only fair quality when canned.

TABLE V.
PEAS.

Variety	Canned			Frozen		
	Colour	Texture	Flavour	Colour	Texture	Flavour
Dwarf Champion ..	6·3	6·6	5·6	9·2	7·1	7·7
Foremost	5·8	7·0	4·9	8·3	8·3	7·4
Lincoln	6·1	7·0	6·1	9·6	7·7	7·6
Petit Pois	5·2	7·0	5·1	7·3	7·3	6·7
Thomas Laxton ..	4·9	6·3	5·6	8·9	8·4	8·9
Average ..	5·7	6·8	5·5	8·7	7·8	7·7

Foremost was not rated quite so highly when frozen as in the previous season, but was still above the average for colour and texture and of quite good flavour, but the canned product was poor.

Lincoln when frozen was of exceptionally good colour and quite good texture and flavour; in comparison the canned product was of only fair quality but it was rated the highest of the series for flavour. In previous tests the colour of the frozen peas was only fairly good.

Petit Pois were of only fairly good colour and flavour when frozen and only fair when canned. The results of tasting this variety have varied considerably in the three seasons it has been tested. The stage of maturity seems more critical with this than with most varieties.

Thomas Laxton peas froze very well, as previously, but the canned product was the poorest of the series, although in previous tests this variety had been fairly good when canned.

Processes.

The frozen peas were again greatly superior to the same variety when canned for colour and for flavour, and also of better texture. The green pea is one of the most successful vegetables for freezing.

Spinach.

Three varieties of spinach were tested for the first time in these trials. The leaves were well washed, weighed into 3 oz. lots and put into 3 quarts of boiling water in successive batches. The water re-boiled within ½ min.; it was boiled a further 2½ min., and the leaves drained and cooled in ice-cold water. The quantity of scalded spinach per can was 12 oz. with 16 fl. oz. of 2% brine. The results after 5 months' storage are given in Table VI.

Varieties.

Giant Savoy Leaf was unusually good in colour, of very good texture and good flavour when preserved by freezing. The colour of the canned spinach

TABLE VI.

SPINACH.

Variety	Canned			Frozen		
	Colour	Texture	Flavour	Colour	Texture	Flavour
Giant Savoy Leaf ..	4·6	8·1	7·1	9·4	8·6	7·7
Perpetual	4·4	7·3	2·9	8·2	7·3	7·5
Prickly New Giant ..	4·7	7·8	7·4	9·4	8·2	7·3

was poor, but the texture and flavour nearly as good as in the frozen product.

Perpetual was quite good in all qualities when frozen, but considered very poor and unacceptable when canned, chiefly due to the " foreign " flavour.

Prickly New Giant was similar in all qualities to the Giant Savoy Leaf when canned and when frozen.

Processes.

The difference in the colour of the canned and frozen products was outstanding : all the frozen spinach being rated above the average, whereas in comparison the canned varieties were considered too poor to be acceptable. The texture of the frozen spinach was slightly better than or similar to that of the canned product. The flavour of all the frozen varieties was quite good, but that of one canned variety was considered objectionable.

Discussion.

The vegetables tested in these trials have all proved satisfactory for freezing, whereas many are, in comparison, very poor when canned.

The excellent colour retention in all the green vegetables preserved by freezing is perhaps the outstanding feature, whereas the canned products in this respect, in spite of the use of artificial colour as in commerical practice, are very disappointing. The colour of the carrots was retained well in both methods of preserving.

The differences in texture were not great, and varied more with the variety than with the method of processing, except with the mushrooms. Scalding the mushrooms before freezing, as is the practice for vegetables, produced a product which was very leathery in texture and could not be softened by subsequent cooking. On the other hand, no pre-treatment, or heating in a small amount of fat (sauté) before freezing, greatly improved the texture : that of the bottled mushrooms was satisfactory.

The flavour of the vegetables after freezing was generally satisfactory, and usually better than that of the corresponding variety when canned, but in this respect the differences in variety were noticeable.

There were slight differences in the quality of some of the peas and beans when compared with the same varieties preserved in 1950 or 1951, but these were not great, and may have been due to some variations in maturity when the vegetables were preserved.

Summary.

The results of preserving 18 varieties of vegetables by canning and freezing show that the latter method gives a product which is outstandingly preferable in colour, and generally better in flavour than the canned one. An improved method of freezing mushrooms is given.

Acknowledgments.

We are indebted to Miss M. Hooper for her help in preserving the vegetables and to all the members of the tasting panel who so regularly helped with the judging.

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METEOROLOGICAL RECORDS, 1952

Month	Air Temperature (°F.)							Rainfall (″)			Sunshine (hours)			Soil Temperatures at 09.00 G.M.T.		
	Means		Means of A & B	Dif. from average	Max.	Min.	No. of ground frosts	Total	Dif. from average	Most in a day	Total	Dif. from normal	Daily average	4″	8″	24″
	A Max.	B Min.														
January	44·4	32·9	38·6	—2·0	52	22	23	3·24	—0·31	0·68	76·1	+28·2	2·46	37·1	37·9	42·2
February	45·5	32·7	39·1	—1·6	51	25	22	0·76	—1·78	0·26	80·6	+13·5	2·78	36·3	37·0	39·8
March	51·0	39·3	45·1	+1·2	58	29	9	2·98	+0·64	0·59	89·1	—33·5	2·87	43·6	43·8	45·1
April	57·9	40·8	49·3	+1·5	73	28	9	1·81	—0·44	0·30	158·3	—1·1	5·28	47·9	47·2	47·9
May	65·1	47·1	56·1	+3·1	79	40	0	4·28	+1·76	1·38	199·7	+ 4·4	6·44	56·9	55·9	56·0
June	67·0	50·5	58·7	+0·3	81	40	0	3·94	+1·80	2·21	206·8	— 5·9	6·89	61·7	59·9	61·0
July	71·5	54·5	62·9	+1·2	88	43	0	0·77	—2·49	0·33	206·2	+10·1	6·65	66·3	65·2	64·1
August	69·0	54·2	61·6	+0·5	76	44	0	6·16	+2·75	1·47	160·8	—24·9	5·19	62·1	61·7	63·4
September	59·8	44·8	52·3	—5·0	68	34	6	3·23	+0·16	1·15	128·0	—13·4	4·27	52·9	53·5	58·6
October	56·0	42·8	49·4	—1·7	62	30	7	4·71	+0·83	1·08	101·9	+ 1·1	3·28	47·3	48·5	52·8
November	45·6	35·5	40·5	—4·2	57	24	15	4·23	+0·68	0·84	67·3	+10·2	2·24	40·0	41·6	47·6
December	45·2	33·2	39·2	—2·2	54	20	21	2·24	—1·55	0·51	66·0	+19·9	2·13	36·8	37·7	41·2
Totals or Means			49·4	—0·7			112	38·35	+2·05		1540·8	+ 8·6	4·21	49·1	49·1	51·7

G. E. Clothier.

EXPERIMENTAL METHODS FOR THE STUDY OF MICRONUTRIENT DEFICIENCIES IN PLANTS GROWN IN SAND CULTURE.

By E. J. HEWITT.
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A progress report (1) summarised the methods devised for the study of iron, manganese and boron deficiencies up to 1946. Full technical details and alternative procedures for these, and later refinements dealing with copper, zinc and molybdenum deficiencies with special reference to large scale installations have been given in a recent book (2), together with an extensive review of relevant work elsewhere. This paper summarises the minimum requirements recommended for reproducible production of severe deficiency status in sand culture for each of the known plant micronutrients. The materials and their preparation are conveniently considered under five headings : containers, sand, water, nutrient reagents and management, and the micronutrients may be grouped for some purposes as follows : (*a*) iron, manganese, boron ; (*b*) copper, zinc ; (*c*) molybdenum.

Methods.

Containers : New horticultural clay pots with a central drainage hole and provided with five coats of pure bitumen solution are satisfactory for group (*a*). Slightly more severe deficiencies may be obtained with some crops if pyrex glass or polythene containers are used and these are essential for work with groups (*b*) and (*c*). Sand is retained with acid-extracted glass wool and a watch glass laid over the drainage hole. Glass containers are supported on waxed wood stands.

Sand : Sand is obtained from Leighton Buzzard, Bedfordshire. It is selected to grade between 20 and 60 I.M.M. mesh and comprises at least 99·8% silica. It is purified with steam and a mixture of 16–18% hydrochloric and 1% oxalic acids in a specially designed apparatus obtainable commercially (2). Full operational details are described elsewhere (2). For elements in group (*a*), 1 or 2 days treatment are satisfactory. For group (*b*) an additional day with alkaline reagents is included, and for group (*c*) 3–4 days with acid and 1 with alkali are normally recommended. After treatment in the apparatus the sand is leached with 20 l. water in twice daily 1 l. amounts followed by 12 l. of suitably purified 0·02 M.$Ca(NO_3)_2$ solution. The leaching is necessary to remove free and exchangeable acid and the soluble impurities, and it is more effective when the containers are filled with sand in the presence of surplus water (2).

Water : Water is obtained by purification of rain water with ion-exchange resins. The sequence comprises three columns of Permutit Zeocarb 215 alternating with two columns of Deacidite E and is completed with a column of Biodeminrolit mixed-bed resin in an all-pyrex glass container measuring 27 × 5 in. For one regeneration, this equipment will provide at least 10,000 l. of water comparable to glass distilled water as shown by the illustration in

Plate XIII at a rate of 10 l. per hour. For elements in group (*a*) water of satisfactory quality is led out immediately before the Biodeminrolit column and an additional 15–20,000 l. are obtained in this manner. Water used for work on elements in groups (*b*) and (*c*) is obtained from the Biodeminrolit column. Full technical details for regeneration of the resins, lay-out and methods of automatic operation and storage and routine tests of purity have been described (2).

Nutrient reagents : These require purification for reproducible results. For removal of iron and manganese alkaline calcium phosphate precipitation (3) in the presence of bicarbonate (1, 2) is effective. For group (*b*) this treatment is repeated if necessary for heavily contaminated salts and is followed by extraction with dithizone in carbon tetrachloride at pH 5·5–6 until there is no colour change at 0·002% concentration with solutions of 1–2 molar concentration. Removal of boron in group (*a*) is most readily achieved by recrystallisation. This is done twice or three times according to purity (1, 2) and an equal volume of purified alcohol is added to the mother liquor after cooling to 0° C. Yields attain 85–90% in this manner. Molybdenum is removed as sulphide by co-precipitation with copper sulphide (4, 2). The process is preferably repeated, unless analytical reagent grade salts are first recrystallised as described. Calcium nitrate is always prepared from A.R. calcium carbonate and nitric acid (1, 2) before the purification treatments.

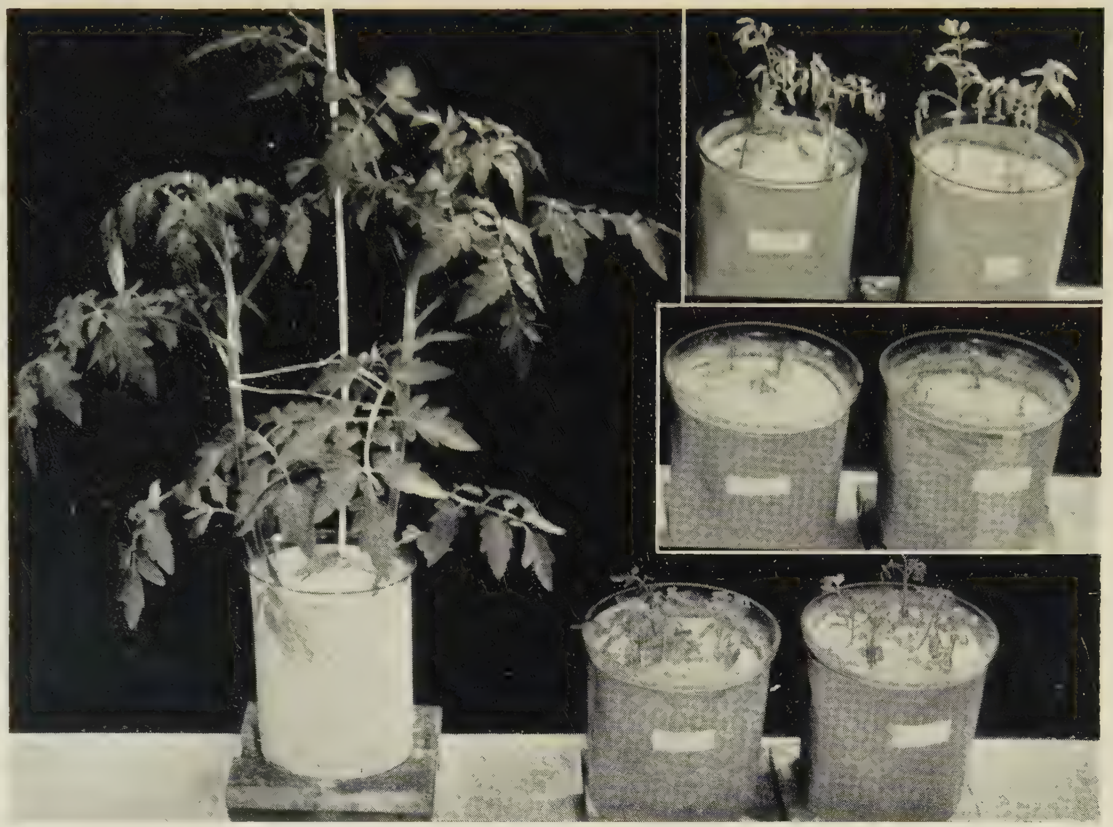
Iron compounds are purified by hydrochloric acid-ether extraction of ferric or ferrous salts combined with sulphide treatment, and detailed procedures for highly purified ferric citrate have been described (2). The treatment of iron compounds is regarded as an important requirement, especially for groups (*b*) and (*c*) and for manganese deficiency. Micronutrient salts of reagent grade are not further purified, when used at normal levels.

The usual complete nutrient composition expressed as mg.eq./l. is as follows : NO_3^-, 15 ; PO_4^{---}, 4 ; SO_4^{--}, 3 ; Ca^{++}, 10 ; K^+ 5 ; Mg^{++}, 3 ; Na^+, 1·33 ; Fe^{+++}, 0·3 ; Mn^{++}, 0·02 ; Cu^{++}, 0·002 ; Zn^{++}, 0·002 ; B^{III}, 0·1 ; Mo^{VI}, 0·012 ; Ga^{+++}, 0·0002 ; Co^{++}, 0·0002 ; Ni^{++}, 0·0002 ; Al^{+++}, 0·001. Nitrogen levels may be raised to 20–25 mg.eq./l. for brassica crops. Ammonium sulphate may be used without injury by mixing about 75 g. calcium carbonate in the upper 4 in. of the sand. The calcium carbonate is prepared from purified reagents if required (2). The solutions are prepared by dilution of separate stock concentrates and intermediate stock mixtures (2).

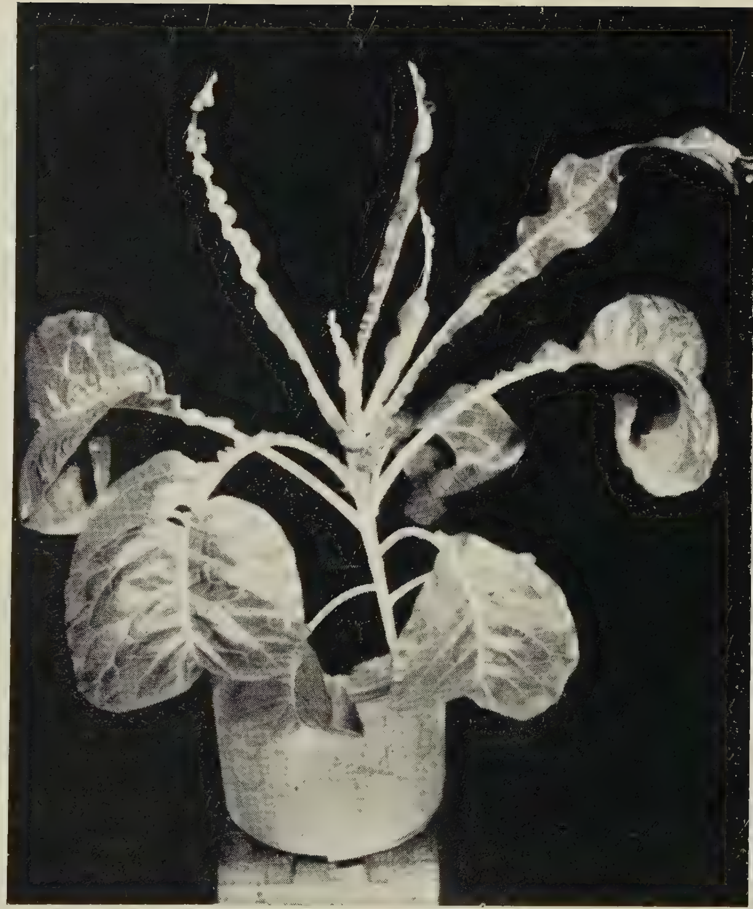
Management : Seeds are sown direct in the sand and seedlings thinned at intervals as required. Some brassicas, e.g. cauliflower, are better transplanted from purified sand. Seed saved from a previous generation grown under deficiency conditions may be required to produce severe deficiency in large seeded legumes of elements in groups (*b*) and (*c*) (5). Nutrient solutions are applied from 5 l. pyrex glass bottles for groups (*b*) and (*c*) and from glazed stone jars for group (*a*). They are carefully poured on to the surface of the sand and excess drains freely to waste. Solution is given at daily or longer intervals according to water uptake. Water only is given once weekly at the weekends. Overhead screens of cellophane or thin perspex sheet supported on light frames substantially reduce contamination by dust, disintegrated paint, or minor roof leakage and condensation.

Particular care is taken in the handling of all containers and solutions and in lay-out to avoid chance contamination by splashing, drip from bottles,

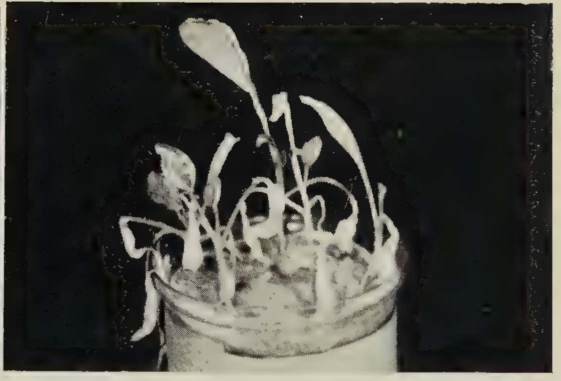
PLATE XIII

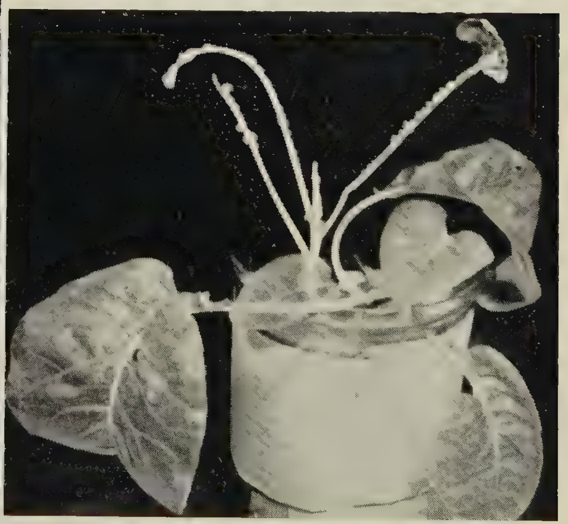


Comparison of water supplies for the production of micronutrient deficiencies in plants grown in sand culture.
Left : tomato plants grown with complete nutrient solution.
Right : top, molybdenum deficiency—middle, zinc deficiency—bottom, copper deficiency.
In each pair the left hand pot was given rain water purified by Permutit ion exchange resins, and the right hand pot was given glass distilled water.



(a) Whiptail in cauliflower grown in sand culture with ammonium sulphate and molybdenum deficiency.





(b) Molybdenum deficiency in cauliflower in sand culture grown with nitrate and extra sodium citrate.

(c) Whiptail in cauliflower grown in sand culture with hands and incidental equipment. These precautions and all attempts to eliminate contamination are additive in effect and contribute materially to the success and consistency of results.

The methods described here have been applied successfully to the production of severe deficiencies of the micronutrient elements in a range of about forty crop plants.

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THE EFFECTS OF SOME DIFFERENT NITROGEN SOURCES ON THE MOLYBDENUM REQUIREMENTS OF CAULIFLOWER PLANTS GROWN IN SAND CULTURE.

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Recent work (1, 2) has indicated that higher plants may still require molybdenum when grown with adequate nitrogen supplied in the ammonium form in addition to nitrate. Results obtained by Agarwala (3) in a large scale experiment intended to test the molybdenum requirements of cauliflower grown with several nitrogen sources showed that molybdenum deficiency, as judged by the production of Whiptail symptoms (4) and growth reduction, could be induced irrespectively of whether nitrogen was given as nitrate, nitrite, ammonium compounds or urea. This paper describes results of a second experiment in which confirmatory results and additional information were obtained.

Methods.

The experimental methods used have been fully described elsewhere (5).

The nitrogen sources given were as follows : nitrate, nitrate + calcium carbonate, nitrate + citrate, nitrate + malate, nitrate + sucrose, nitrate + arginine, nitrite, nitrite + citrate, nitrite + malate, ammonium sulphate + calcium carbonate, ammonium citrate, ammonium malate, ammonium nitrate, ammonium nitrite, urea, urea + citrate, urea + malate glutamic acid. Each source provided 15 m M l. nitrogen except where arginine was given as an additional 2 m M Nitrogen. All reagents, except arginine, were purified from molybdenum (5) and resin purified rain water was used for the nutrient solutions.

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Treatments were replicated in 2 blocks.

Results.

Yields : Data in Table I show that growth was reduced by molybdenum deficiency with all sources of nitrogen. The depression was most marked in the presence of nitrate, especially when citrate, malate or sucrose were also given. There was no interaction between these supplements and the other nitrogen sources. The apparently beneficial effect of arginine may have reflected a slight molybdenum impurity as this compound was not purified, but the analytical data suggest that no significant introduction of molybdenum had occurred.

Yields were also decreased in the normal molybdenum series by many nitrogen sources as compared with nitrate. The effects of molybdenum deficiency were, however, relatively much greater.

Visual symptoms : Wilting and withering of foliage caused by molybdenum deficiency occurred *only* in the presence of nitrate, including ammonium nitrate. Mottling was also characteristic of nitrate treatments but occurred to a lesser extent in some plants given nitrite. Whiptail effects developed in *all* molybdenum deficiency treatments, including nitrite, from which they were absent in the previous experiment, and glutamic acid which was not previously tested. Whiptail was least prevalent in the nitrite treatments especially ammonium nitrite, but was accentuated in the citrate or malate supplements. These supplements also greatly increased the severity of the "primary" deficiency symptoms of mottling, wilting and withering (6) shown in the nitrate treatments and most plants given these combined treatments did not progress to the secondary Whiptail stages. There were no obvious interactions with the supplements in the ammonium or urea treatments.

No visual abnormalities occurred in the normal molybdenum series in any of the different nitrogen treatments. The effects of some treatments are shown in Plate XIV.

Chlorophyll content : Total chlorophyll content extracted by 85% acetone is shown in Table I. The concentration was greatly decreased by molybdenum deficiency only in the presence of nitrate : no decrease occurred in the presence of nitrogen given solely as ammonium compounds or as urea. A slight decrease occurred in the presence of nitrite. Addition of citrate or malate in the presence of nitrate decreased chlorophyll more than with nitrate alone. This interaction did not occur with the other nitrogen sources.

Nitrate content : Nitrate, as determined by phenol disulphonic acid (7), accumulated to high concentrations in foliage only in molybdenum deficient plants given nitrate. Somewhat lower levels were found in plants given nitrate + nitrite and ammonium nitrate. This was consistent with the decreased nitrate supply in these treatments. Decreased nitrate accumulation in the arginine treatment may have reflected slight molybdenum contamination. The citrate, malate and sucrose supplements greatly accentuated nitrate accumulation when nitrate was given.

TABLE I.

EFFECTS OF MOLYBDENUM SUPPLY AND NITROGEN SOURCE ON YIELD, G. DRY WT.; CHLOROPHYLL CONTENT, MG. PER 100 G. FRESH WT.; NITRATE CONTENT, PER CENT IN FRESH WT. AND MOLYBDENUM CONTENT, P.P.M. IN DRY FOLIAGE.

Nitrogen source and supplements	Yields		Ratio per cent	Chlorophyll		Ratio per cent	Nitrate		Molybdenum Content	
	0·00001 p.p.m. Mo	0·05 p.p.m. Mo		0·00001 p.p.m. Mo	0·05 p.p.m. Mo		0·00001 p.p.m. Mo	0·05 p.p.m. Mo	0·00001 p.p.m. Mo	0·05 p.p.m. Mo
Nitrate	22·6	218·2	10·4	111·0	208·7	53·1	0·485	0·053	0·064	3·83
Nitrate + $CaCO_3$	39·1	160·9	24·2	124·7	234·8	51·1	0·502	0·058	0·028	8·84
Nitrate + Citrate	4·9	131·7	3·7	49·9	191·2	26·2	1·375	0·045	0·043	1·30
Nitrate + Malate	1·9	83·2	2·3	49·6	194·9	25·5	1·756	0·072	0·065	3·25
Nitrate + Sucrose	7·0	101·9	6·9	66·6	170·5	39·0	0·960	0·036	0·011	3·60
Nitrate + Arginine	116·2	202·3	57·3	116·0	227·0	51·1	0·332	0·086	0·011	4·19
Nitrate + Nitrite	81·3	155·6	52·2	183·7	223·7	82·2	0·290	0·047	0·033	3·61
Nitrite	54·7	145·2	37·6	188·5	215·1	87·5	0·051	0·046	0·014	5·00
Nitrite + Citrate	27·8	112·0	24·8	190·9	230·8	80·8	0·024	0·014	0·095	4·01
Nitrite + Malate	36·3	99·3	36·5	177·5	190·0	93·3	0·033	0·017	0·054	6·47
Ammonium nitrate	19·1	127·0	15·0	129·5	253·5	51·2	0·214	0·029	0·065	2·60
Ammonium nitrite	24·8	56·4	49·2	272·4	215·9	126·2	0·028	0·005	0·044	2·23
Ammonium sulphate + Calcium carbonate	77·2	177·6	43·4	207·1	198·4	104·2	0·027	0·046	0·072	2·60
Ammonium citrate	50·1	70·7	70·8	233·5	159·6	146·0	0·024	0·043	0·062	1·14
Ammonium malate	29·1	95·9	30·4	228·8	194·6	117·7	0·016	0·023	0·053	3·32
Urea	38·5	85·4	45·1	202·3	235·5	85·8	0·008	0·008	0·028	4·56
Urea + Citrate	50·1	129·1	38·8	198·8	190·4	103·5	0·029	0·018	0·070	4·88
Urea + Malate	45·7	87·9	51·8	191·1	219·2	87·1	0·018	0·031	0·024	5·00
Glutamic acid	44·5	68·9	64·6	230·9	190·7	116·8	0·035	0·004	0·100	8·46
Mean	40·6	123·2		166·5	207·6		0·326	0·036	0·049	4·14

All plants appeared to contain some nitrate but its origin and the possibility of artifacts arising in the determination require further study.

Ascorbic acid : A few determinations were made to confirm that depression of ascorbic acid concentration was caused by molybdenum deficiency irrespective of nitrogen source. The molybdenum effect was not related to nitrogen source and this confirms more extensive data obtained in the previous experiment (3). The rapid response to injection by molybdenum (8) was also repeated.

Molybdenum content : Dried foliage was analysed for molybdenum using the method of Piper and Beckwith (9). Results are given in Table I. They show that where molybdenum was omitted the content was generally between 0·05 and 0·1 p.p.m. Calcium carbonate itself did not apparently contribute any molybdenum as judged by comparison of nitrate with or without calcium carbonate, and the plants given ammonium sulphate were obviously acutely molybdenum deficient. Plants given a normal supply of molybdenum contained from 1·8–8·5 p.p.m. Mo. Concentrations were possibly slightly higher in the presence of nitrite, urea or glutamic acid than with ammonium or nitrate treatments. Inclusion of citrate or malate did not generally have any notable effects on molybdenum concentration.

Discussion.

The results obtained here are consistent with the view that molybdenum is required by the cauliflower when grown with a wide range of nitrogen sources. The possibility that each of these was mainly fully nitrified to nitrate prior to absorption is not disproved at this stage but it is regarded as highly unlikely because, (*a*) nitrate accumulation was only pronounced in the nitrate treatments, (*b*) initially sterile and uninoculated sand cultures probably remain free from nitrification for relatively long periods (5), (*c*) effects on Whiptail development, ascorbic acid content, reducing activity (10) and growth were independent of nitrogen source and would require to be attributed to nitrate uptake that could not be detected by other means, whilst effects on arginine content (11) and on chlorophyll production noted here comprise responses associated with certain of the non-nitrate treatments, or nitrate respectively.

The striking effects of citrate and malate in the presence of nitrate and the less but still noticeable effects in the presence of other nitrogen sources was not generally related to any effect on molybdenum content, and internal factors may have been involved in these interactions.

Speculation on the role of molybdenum would be premature at this stage but it appears that either nitrate reduction represents only one of several roles or that this process is incidental to a more fundamental aspect of metabolism. Chlorophyll production, however, on the other hand, is one of the few aspects of metabolism so far investigated for which a molybdenum requirement is not evident, except when nitrate assimilation is impeded by molybdenum deficiency.

Acknowledgments.

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THE EFFECTS OF SEED RESERVES ON THE PRODUCTION OF DEFICIENCIES OF COPPER, ZINC AND MOLYBDENUM IN SOME LARGE-SEEDED LEGUMINOUS CROPS.

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Previous work (1) showed that molybdenum deficiency symptoms were not readily produced in crops such as beans and peas grown in sand cultures. It was thought likely that seed reserves might account at least in part for this difficulty, and seed was saved from plants grown in deficiency and complete-nutrient sand cultures for comparison with results obtained with commercially raised seed. Seed was saved from plants deficient also in copper or zinc as this material was available at the time. Whilst this work was in progress Meagher, Johnson and Stout (2) were led by similar considerations to the conclusion that seed history could mainly account for the low susceptibility to molybdenum deficiency of some of the large-seeded legumes tested also in our experiments.

Experimental.

Methods.

The sand culture methods used have been described in detail elsewhere (3) together with details of nutrient purification and water production. The only point to merit comment here is that all water supplies used for this work were obtained by purification of rain water by ion exchange resins (3) which included a final column of Permutit Biodeminrolit mixed-bed resins held in a pyrex glass column measuring 27 in. × 5 in. and this delivered water comparable to glass distilled water at 10 l. per hour (3, 4, 5).

The complete nutrient solution was similar to that described before (3).

Results.

The yield data in Table I show the effects on growth of using seed saved from sand cultures compared with use of seed obtained commercially. There was evidently a growth depression associated with the use of seed obtained from sand cultures irrespective of whether complete nutrient or deficiency treatments were concerned in the origin of the seed and in the maintenance of the plants raised therefrom.

TABLE I.

EFFECTS OF SEED ORIGIN ON GROWTH OF LARGE-SEEDED LEGUMINOUS CROPS IN SAND CULTURES (MEAN YIELDS G. DRY WT./PLANT).

Crop	Origin of Seed*	Treatments			
		Complete nutrient	Copper deficiency	Zinc deficiency	Molybdenum deficiency
Pea	Commercial 1952	26·1	—	7·2	24·0
	Sand culture	23·0	—	4·6	9·8
Dwarf bean	Commercial 1952	73·1	31·0	11·1	118·3
	Sand culture	41·9	1·1	4·6	32·3
Runner bean	Commercial 1952	117·8	49·8	39·8	147·3
	Sand culture	—	12·2	12·5	22·8
Broad bean	Commercial 1952	66·2	46·7	26·8	55·6
	Sand culture	50·0	2·9	12·5	25·6

*Seed obtained from plants grown in sand cultures was re-sown in cultures of the same treatment as that from which it was harvested.

The growth depression associated with seed saved from the deficiency treatments was much greater however and clearly showed the importance of seed history and reserves on the incidence of copper, zinc and molybdenum deficiencies in these particular crops. This conclusion was indicated equally clearly from comparison between yields with sand culture or commercial seed for each deficiency treatment, and from comparison of the yield ratios for each deficiency in relation to the complete nutrient for the two types of seed. With the exception of runner bean the relative effects of impoverished seed reserves decreased in the order : copper, molybdenum, zinc and the effect was always least for zinc.

Visual symptoms were increased in severity or were apparent for the first time when the impoverished seed was used.

Copper deficiency in dwarf bean was qualitatively similar to effects described before (4). Similar necrotic areas in dark green leaflets and scorching were seen in runner beans. Elongation was checked and death of the growing point occurred. Broad beans had erect, inrolled or folded younger leaflets which became chlorotic and finally blackened. Death of the growing point occurred. Old leaves slowly faded.

Zinc deficiency symptoms were generally more severe than those seen in 1951 (4) and were further accentuated by depleted seed reserves. Dwarf beans had faded and orange tinted leaflets. These showed pronounced downward curvature, with necrosis of veins visible on the lower surface and marginal scorching. Axillary shoots were markedly rosetted in habit. Stem elongation of runner beans was greatly checked. Leaflets had characteristically wavy margins and were unsymmetrical in a manner resembling the " sickle leaf " disorder of cacao (6). Leaflets also bore rounded, dark-green spots irregularly distributed on a generally faded chlorotic background. Broad beans showed characteristic reduction of stem internode length. Leaflets were relatively narrow in outline and wavy along the margins, and sharply reduced in size towards the stem apex. These features resulted in a sharp triangular outline. Young leaves were chlorotic and finally necrotic. Peas had dull green foliage which became necrotic and scorched in the mid-stem regions ; the few pods bore necrotic surface lesions.

Molybdenum deficiency resulted in wilting, scorching and withering of older leaves in all crops. This was preceded by pronounced chlorosis and diminished size in runner bean leaflets. Dwarf beans showed no symptoms until 8 weeks after they were evident in runner beans which responded 3–4 weeks after sowing.

The appearance of molybdenum deficiency symptoms was relatively sudden and often developed in 1–2 days. This confirmed the observations of Meagher *et al.* (2).

Molybdenum symptoms only appeared in plants raised from the depleted seed saved from the previous sand cultures. In copper and zinc deficiencies symptoms appeared in plants raised from both sets of seed but differed in severity.

Some typical effects in this work are shown in Plate XV.

The analytical data in Table II show certain points of interest. The content of copper. zinc or molybdenum was sharply decreased in seed saved from the respective deficiency sand cultures. The differences were extreme for copper and least for zinc. The molybdenum content of runner beans was exceptional in that it was decreased only on a basis of content per seed compared with the commercial seed used in the comparative test. Seed weight was, of course, an additional factor affecting the response. Seed saved from complete nutrient cultures was similar in content of the elements under consideration to seed from commercial origin, except that the molybdenum content of dwarf and runner bean seed saved from the complete nutrient cultures was markedly higher than in commercial seed. Seed obtained commercially in two successive years differed notably in content of the three elements.

The relative concentration of the elements in the normal seed generally decreased in the order zinc, copper, molybdenum.

Analytical data on the foliage of plants grown in these experiments have

TABLE II.

RELATION OF SEED HISTORY TO

CONTENT OF COPPER, ZINC AND MOLYBDENUM AS P.P.M. AND PER SEED.

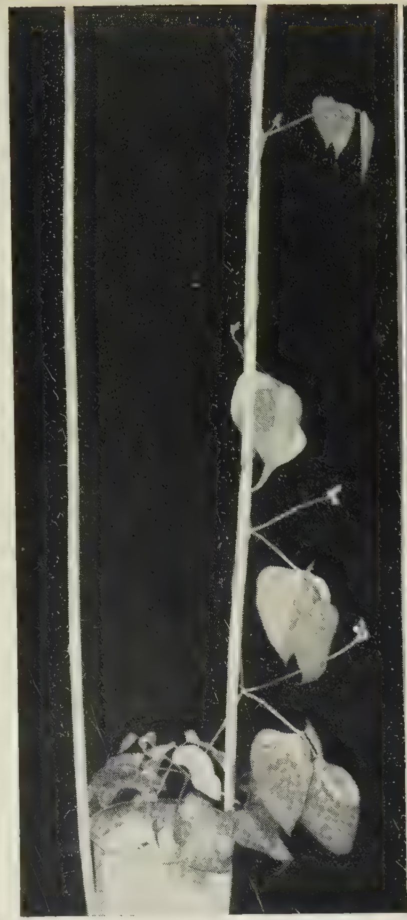
Seed	Origin	Copper		Zinc		Molybdenum	
		p.p.m.	µg./seed	p.p.m.	µg./seed	p.p.m.	µg./seed
Pea	Commercial 1951	8·3	2·6	7·3	2·3	0·91	0·28
	,, 1952	4·2	1·2	24·2	6·0	0·57	0·16
	Complete nutrient sand culture	2·9	0·80	16·9	4·7	3·0	0·83
	Deficiency culture –Cu, *or* –Zn *or* –Mo	—	—	11·5	3·7	0·24	0·07
Dwarf bean	Commercial 1951	3·7	1·8	31·8	15·4	2·3	1·2
	,, 1952	4·5	2·4	25·8	13·8	6·1	3·2
	Complete nutrient sand culture	8·3	4·0	41·7	20·0	6·0	2·9
	Deficiency culture –Cu *or* –Mn *or* –Mo	below 0·04	below 0·01	8·2	3·9	0·11	0·04
Runner bean	Commercial 1951	5·1	3·5	8·2	5·7	0·52	0·36
	,, 1952	2·3	2·4	16·9	17·7	0·19	0·20
	Complete nutrient sand culture	4·8	2·9	29·2	18·0	2·3	1·4
	Deficiency culture –Cu *or* –Zn *or* –Mo	below 0·05	below 0·01	11·5	3·9	0·28	0·10
Broad bean	Commercial 1951	8·6	8·5	14·2	14·1	1·1	1·1
	,, 1952	8·1	16·8	22·5	46·8	3·6	7·5
	Deficiency culture –Cu *or* –Zn *or* –Mo	—	—	—	—	0·13	0·14

been obtained but are not presented in this report. It was found, however, that the origin of the seed was not reflected in the nutrient status of the foliage, as concentrations were not further reduced in plants grown from the depleted seed. The effect of seed reserves was shown on total growth and therefore in total content, and not in concentration. Levels of copper and molybdenum were sharply reduced in the deficiency treatments but zinc concentrations were, relatively, not greatly decreased in spite of the marked growth reduction that occurred.

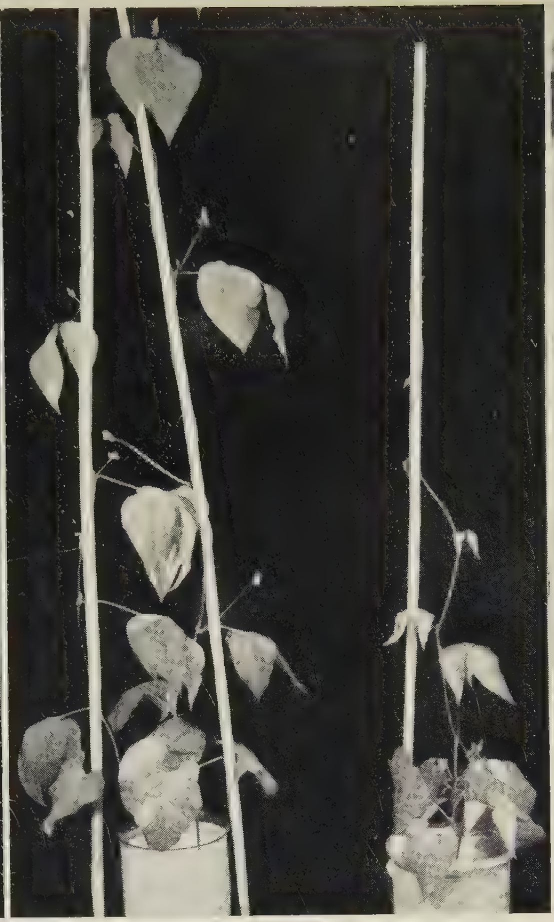
Discussion.

The effect of seed reserves on the incidence of the three deficiencies tested is reasonably clear. The significance of the reduction in yield associated with the use of seed saved from complete-nutrient sand cultures is, however, not understood. It is possible that conditions of saving the seed were unfavourable although all reasonable care was taken and the advice of experienced seedsmen was followed as far as possible. It is known, however, (3) that seed history may affect subsequent growth. The possibility of some other element being depleted cannot be excluded at this stage and the drastic reduction in reserves of copper in particular might have occurred with other essential elements more readily available under field conditions and whose status is not yet recognised.

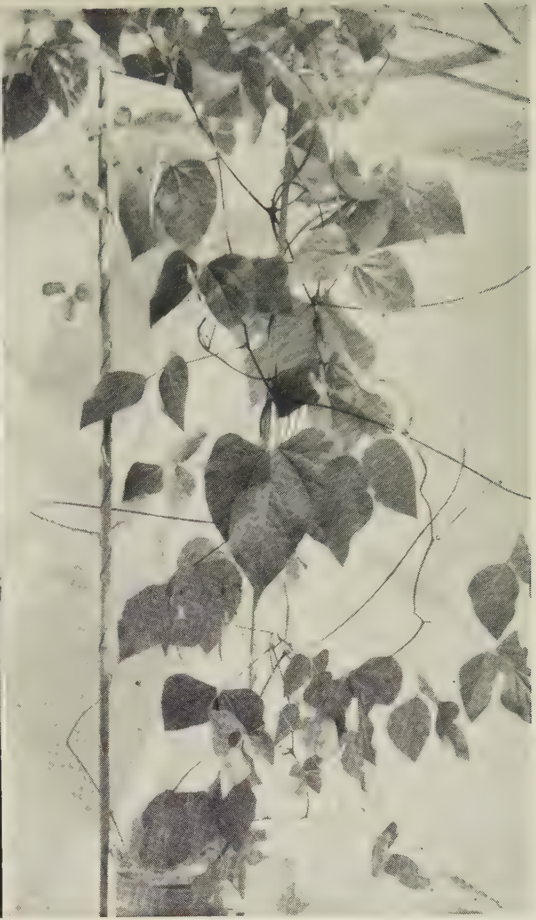
PLATE XV



Copper deficiency in runner bean.
Left : seed from copper-deficient sand culture.
Right : commercial seed.



Zinc deficiency in runner bean.
Left pot : control treatment with complete nutrient :
Left-hand plant : seed obtained from previous complete nutrient treatment.
Right-hand plant : seed obtained commercially.
Right pot : zinc deficiency treatment :
Left-hand plant : seed obtained commercially.
Right-hand plant : seed from previous zinc-deficient sand culture.



Molybdenum deficiency in runner bean.
Left : seed obtained from molybdenum-deficient sand culture
Right : commercial seed.

The effects of copper deficiency on seed reserves noted above showed that the extent to which seeds might accumulate mineral reserves under conditions of restricted supply might differ greatly for different elements, since zinc deficiency resulted in relatively much smaller decreases in zinc content of the saved seed. Molybdenum behaved in an intermediate manner.

It is concluded that both seed origin, and weight as affecting mineral content, may fully account for initial difficulties on the production of molybdenum deficiency in sand cultures in the large seeded leguminous crops. Thus the results of Meagher *et al.* (2) are confirmed and the conclusions of Wilson (7) on the factors affecting the incidence of " Scald " in beans appear fully justified. In addition it may be expected that behaviour of these crops under conditions of copper and zinc deficiencies will also depend on field history in affecting mineral reserves, as concluded already by Piper (8) for cereals. Other examples and their application to culture methods have been discussed elsewhere (3).

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SOME EFFECTS OF METALS IN EXCESS ON CROP PLANTS GROWN IN SOIL CULTURE:

V. EFFECTS OF COPPER ON THE MINERAL STATUS OF OAT, RYE, BARLEY, SUGAR BEET AND KALE.

By **W. A. FORSTER.**

In a previous report (1) the visual symptoms occurring in oat, rye, barley, sugar beet and kale, grown in soils treated with copper sulphate, were described. Chemical analyses of the material harvested from these experiments show that the application of copper sulphate had no significant effect on the iron status of the treated plants, but that the Cu, Mn, K, Ca, Mg and P contents were generally changed. The effects tended to be similar from one crop to another, hence detailed analyses are given for oat only and these are compared with the results obtained with the other crops.

Experimental.

Details of the experiments summarised below have already been described (1).

Expt.	Crop	Soil	m.eq. rates of Cu applied per pot Cu_0 Cu_1 Cu_2 Cu_3	Harvest
E	Oat, var. S84	3 parts sandy loam, plus 2 parts granite chips. pH 5·9	0 12·5 25 50 Applied 4 weeks before transplanting	4 weeks from transplanting, 5 weeks from seed
F	Rye, var. Giant. Barley, var. Plumage Archer	do.	0 5 10 20 Applied 6 days before transplanting	3 weeks from transplanting, 4½ weeks from seed
G	Sugar beet, var. Klein Wanzleben. Kale, var. Borecole	Soil derived from New Red Sandstone, pH 6·4	0 10 20 40 Applied 4 days before transplanting	6 weeks from transplanting, 9 weeks from seed

Rye and barley in experiment F, and sugar beet and kale in experiment G, were arranged in split plot design. Each experiment (E–G) contained 192 six-inch bitumen-painted pots (soil-content 2 kg.) arranged in four randomised blocks in the glasshouse. Sugar beet and kale plants given 40 m.eq. Cu were not sampled because of the high mortality at that Cu level (1).

Dry weights of tops and roots were determined. The dried samples were wet digested with nitric and perchloric acids (3) and analysed for K, Ca, Mg and P (tops and roots), and Cu, Fe, Mn (tops only). Magnesium was determined by titration with ethylenediamine-tetraacetic acid (2); the other nutrients were determined by the methods listed in Paper VI of this series.

Results.

The following units were employed in the graphical and statistical treatment of the results :—

Dry weights : log (1 + dry weight per sampling unit in g.).

Fe : μg/g. dry material.

Cu, Mn : log μg/g. dry material.

K_2O, CaO, MgO, P_2O_5 : % of dry material.

Tables I and II summarise the statistical analyses of dry weights and chemical analyses. These results are also compared in Figs. 1–10.

TABLE I.

SOME EFFECTS OF COPPER ON DRY WEIGHTS AND MINERAL STATUS OF PLANT TOPS.

	Oat	Rye	Barley	Beet	Kale
Dry Wt.	0, 1>2>3 **	0>1>2>3 **	0>2>3 ** 1>3	0>1, 2 **	0>1>2 **
Cu ..	3>2, 1, 0 ** 2>0	3>2, 1, 0 ** 2>0	3>2, 1, 0 ** 2>0	2>1, 0 *	2, 1>0 **
Fe ..	N.S.	N.S.	N.S.	N.S.	N.S.
Mn ..	3>2, 1>0 **	3>2, 1, 0 ** 2>0	3>2, 1, 0 **	2>0 **	2>1, 0 **
K_2O ..	0>1, 2, 3 ** 1>3	0, 1>3 **	0, 1>3 **	0>2 *	0>2 *
CaO ..	N.S.	3>2, 1, 0 **	3>2, 1, 0 *	2>1>0 **	N.S.
MgO ..	3>1, 0 **	—	3>2, 1, 0 *	N.S.	2>0 *
P_2O_5 ..	0>1, 2, 3 **	0>2, 3 **	0>1, 2 *	0>1, 2 *	0>1 **

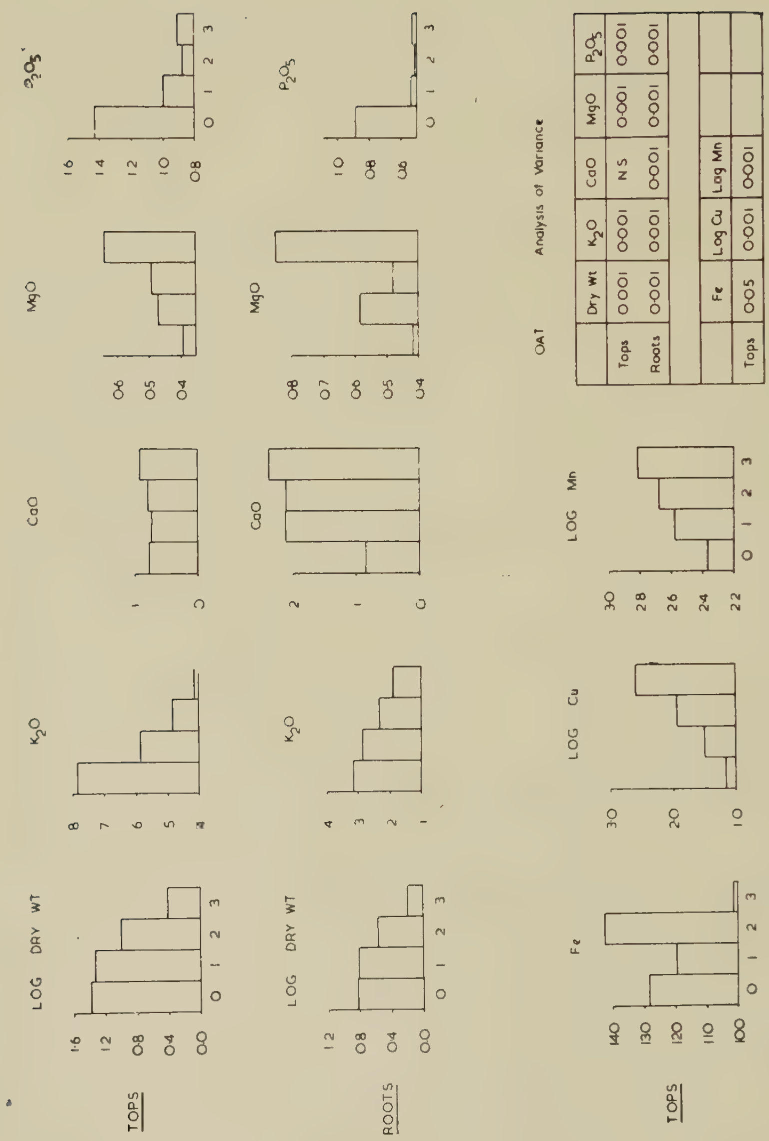
** Significant at *P* = 0·001.
* ,, ,, *P* = 0·01.
N.S. *P*>0·01.
0, 1, 2, 3, signify the respective rates of Cu application (viz. Cu_0, Cu_1, Cu_2, Cu_3).

TABLE II.

SOME EFFECTS OF COPPER ON DRY WEIGHTS AND MINERAL STATUS OF PLANT ROOTS.

	Oat	Rye	Barley	Beet	Kale
Dry Wt.	0, 1>2>3 **	0>2>3 ** 1>3	0, 1>2>3 **	0>2 **	0>1, 2 **
K ..	0>2, 3 ** 1>3	0, 1>3 **	0, 1>3 **	0>1, 2 *	0>2 **
Ca ..	3, 2, 1>0 **	N.S.+	N.S.+	2>0 *	2>0 **
Mg ..	3>2, 1, 0 **	N.S.+	N.S.+	N.S.	N.S.
P ..	0>1, 2, 3 **	0>1, 2, 3 **	0>1, 2 *	0>2 **	0>2 **

** Significant at *P* = 0·001.
* ,, ,, *P* = 0·01.
N.S. *P* >0·05.
0, 1, 2, 3, signify the respective rates of Cu application (viz. Cu_0, Cu_1, Cu_2, Cu_1).
+ Based on data at Cu_0, Cu_1 and Cu_2 only.



OAT Analysis of Variance

	Dry Wt	K_2O	CaO	MgO	P_2O_5
Tops	0·001	0·001	N S	0·001	0·001
Roots	0·001	0·001	0·001	0·001	0·001

	Fe	Log Cu	Log Mn
Tops	0·05	0·001	0·001

Fig. 1. The effects of copper on growth and mineral status of oat. K_2O, CaO, MgO and P_2O_5, as % dry material; Fe, Cu and Mn as (log) μg/g.
Abcissae indicate m.eq. rates of copper application, as follows: $0 = 0, 1 = 12{\cdot}5, 2 = 25, 3 = 50$.

Oat.

In Fig. 1 it is shown that the application of copper sulphate progressively decreased the dry weights of tops and roots and their K_2O and P_2O_5 contents, but increased the MgO contents. The CaO status was increased in roots by the copper treatment, but was unaffected in the tops. The Cu and Mn levels in oat tops were progressively increased by copper treatment whereas the Fe status was significantly affected only by the highest rate of application of copper sulphate, which gave a decrease significant at $P = 0{\cdot}05$.

Oat, rye, barley, sugar beet and kale.

The dry weights of tops and roots of all crops were significantly decreased by the application of copper. A comparison of Figs. 2 and 3 shows that tops were usually more seriously affected than roots. This may be due to an immobilisation of Cu in the roots at the lower rates of Cu application only, for an earlier experiment with oat (1) showed that when this crop was grown in soil treated with increasing amounts of Cu, the copper content increased proportionately more in the tops than in the roots.

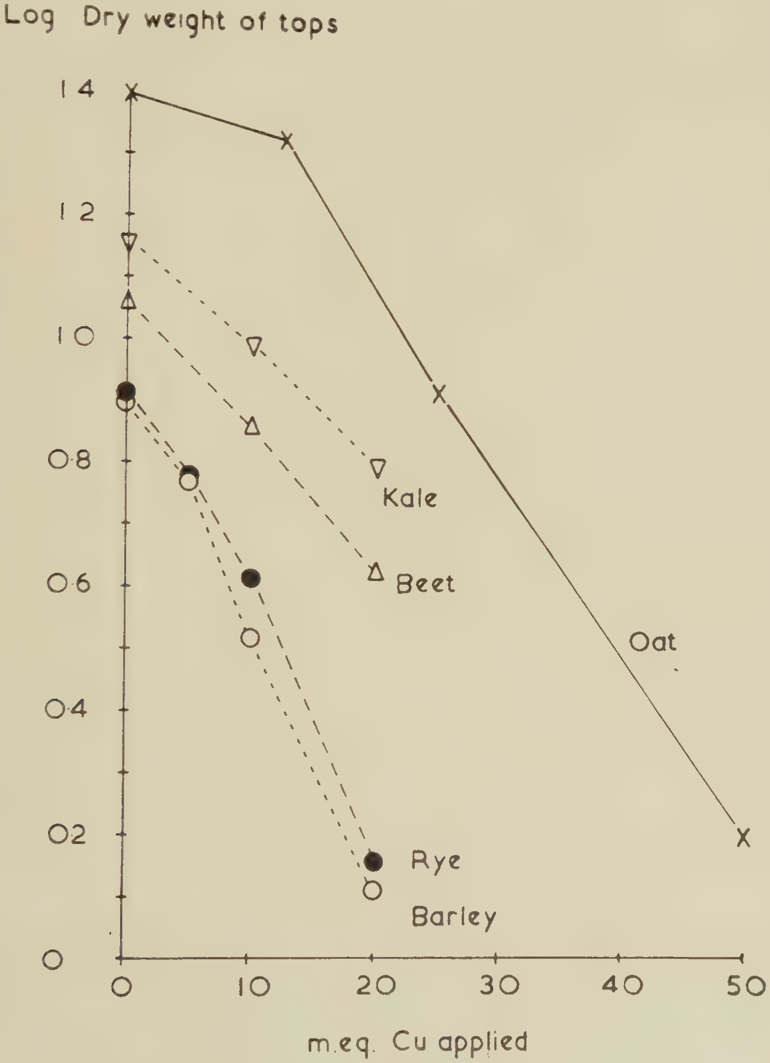


Fig. 2. The effects of copper on the dry weights of oat, rye, barley, sugar beet and kale tops.
Ordinates : log (1 + total dry weight per sampling unit in g.).
Abcissae : m.eq. Cu applied (as $CuSO_4$) per pot.
A sampling unit consisted of 12 pots for oat, and 6 pots for each of the other four crops, respectively.

With each crop the Cu content of tops was progressively increased by copper application as shown in Fig. 4 and Table I.

There was no consistent relation between the Fe status of the crops and the application of copper sulphate (Fig. 5). At the highest rate of Cu application there was an increase in the Fe status of barley and a decrease in the Fe status of oat, both significant at $P = 0{\cdot}05$ only.

The Mn status of all crops was increased by the application of Cu (Table I). The increases occurring in barley and rye were similar in magnitude (Fig. 6); these crops also showed similar increases in Cu status (Fig. 4) and similar decreases in the dry weights of tops (Fig. 2).

Copper application significantly decreased the K_2O status of tops and roots of each crop (Fig. 7, Tables I and II).

CaO status was generally higher in treated plants than in controls (Fig. 8). With oat and kale, however, copper treated plants showed an increase in CaO status of roots but not of tops.

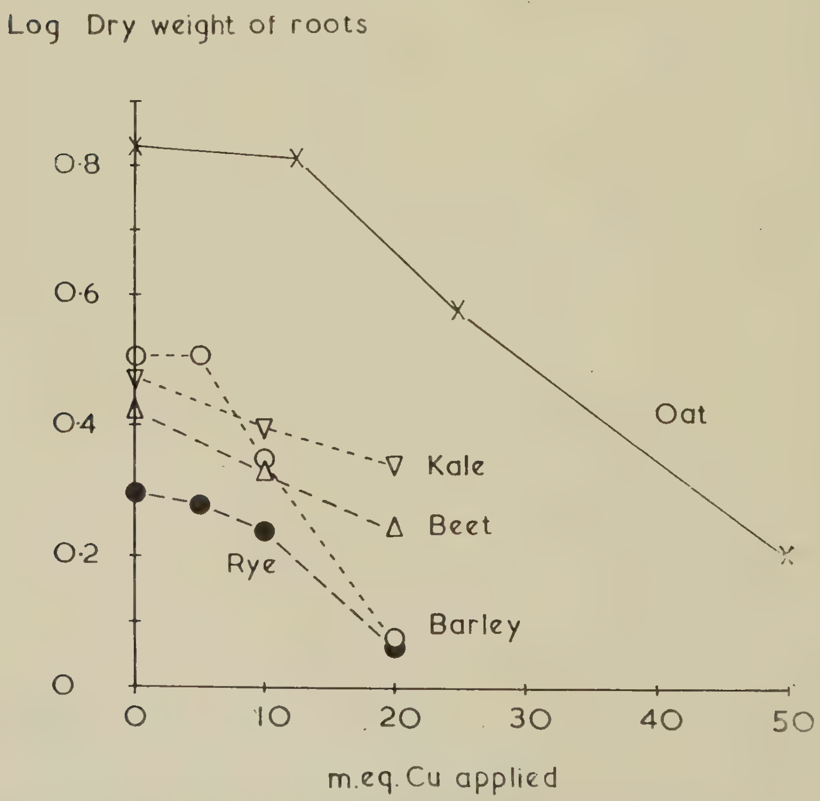


Fig. 3. The effects of copper on the dry weights of oat, rye, barley, sugar beet and kale roots.

Ordinates : log (1 + total dry weight per sampling unit in g.).

Abcissae : m.eq. Cu applied (as $CuSO_4$) per pot.

A sampling unit consisted of 12 pots for oat, and 6 pots for each of the other four crops, respectively.

The full data for MgO are not available, but it appears that the MgO status tends to rise when Cu is applied to the soil (Fig. 9). The apparent effect of Cu on Ca and Mg status may be an indirect one, caused by the reduced K uptake.

In each crop, the P_2O_5 content is decreased at the lowest rate of Cu applied (Fig. 10), but with increasing Cu application P_2O_5 status is either unchanged or in some cases increased. A similar effect has also been observed in another experiment with oat (1).

Correlation coefficients (not reported in this paper), were determined for each pair of nutrients with oat, rye and barley tops. (Cu, Fe, Mn, K, Ca, Mg, P, 21 combinations of nutrients, taken in pairs, i.e. 21 'r' values for each crop). Most of the 'r' values were significant ($P = 0{\cdot}01$ or $0{\cdot}001$) and with such highly correlated systems it is not possible to distinguish between direct and indirect effects of copper on the mineral status of the crops.

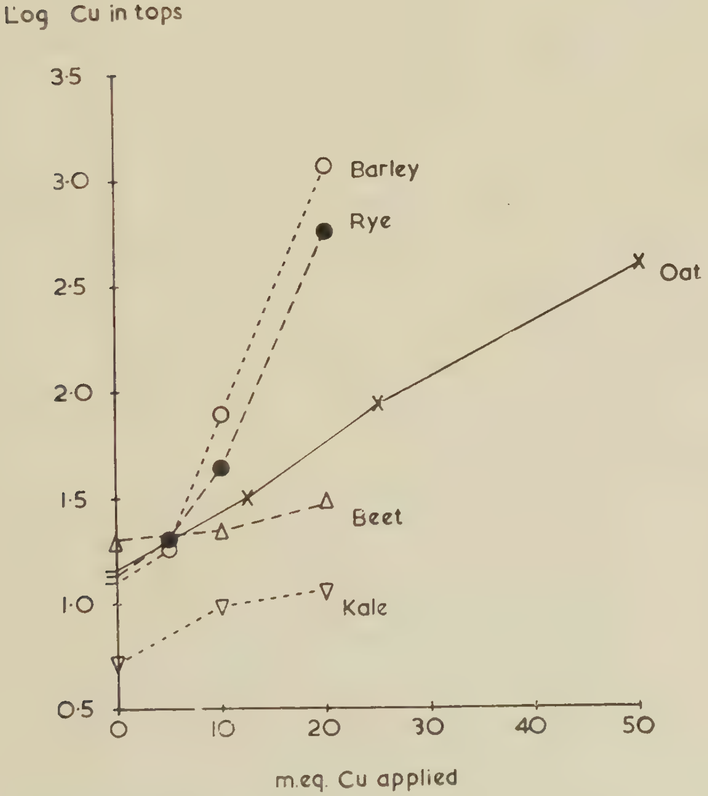


Fig. 4. The effects of copper on the copper status of plant tops.
Ordinates : log (μg Cu/g. dry material).
Abcissae : m.eq. Cu applied (as $CuSO_4$) per pot.

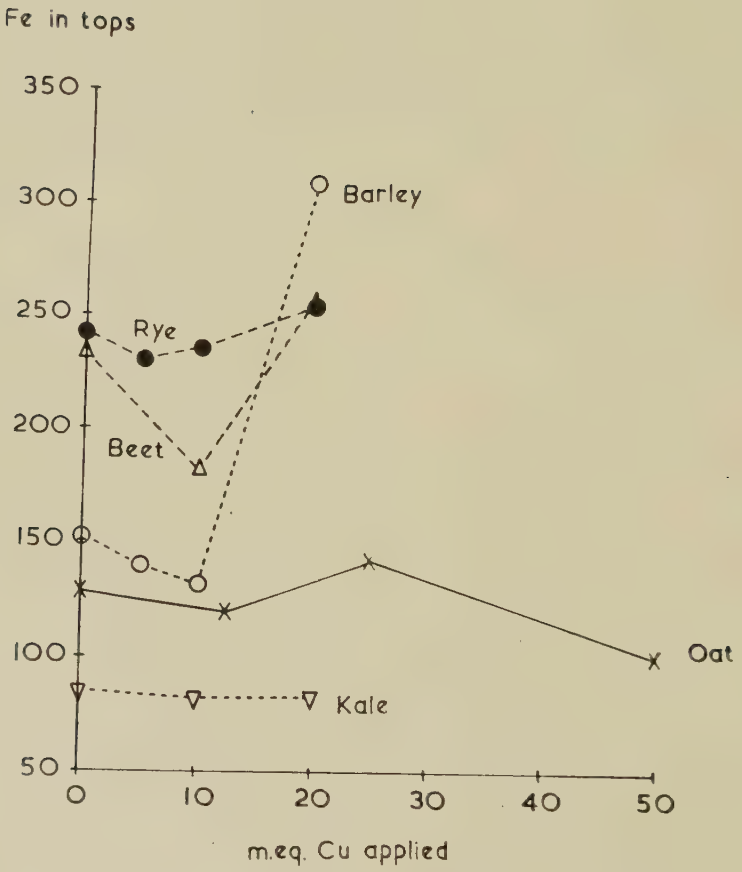


Fig. 5. The effects of copper on the iron status of plant tops.
Ordinates : μg Fe/g. dry material.
Abcissae : m.eq. Cu applied (as $CuSO_4$) per pot.

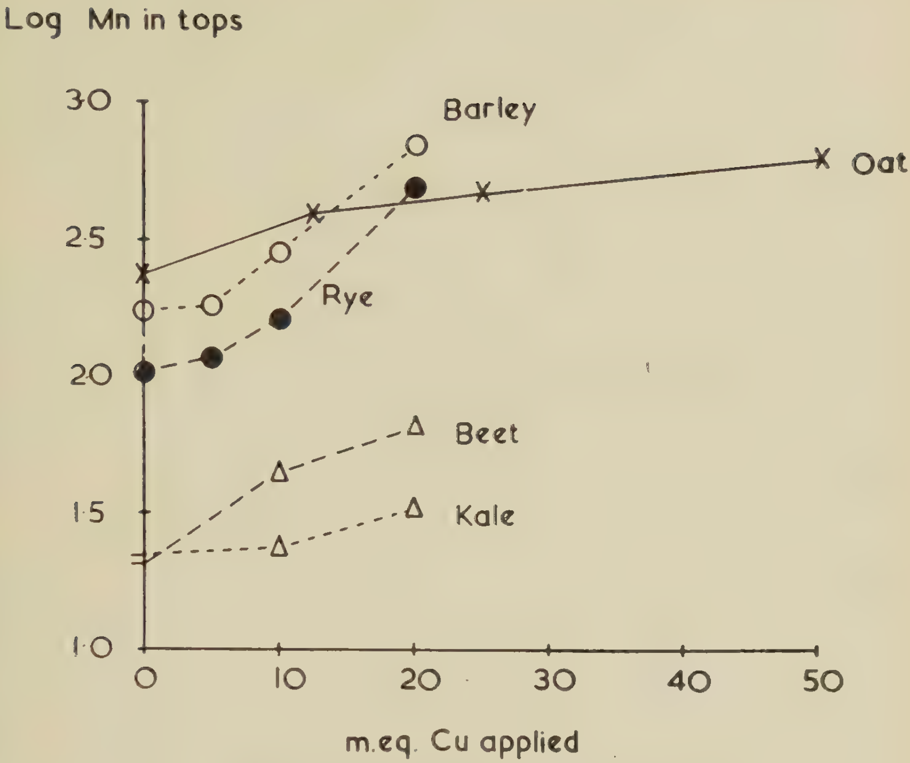


Fig. 6. The effects of copper on the manganese status of plant tops.
Ordinates : log (μg Mn/g. dry material).
Abcissae : m.eq. Cu applied (as $CuSO_4$) per pot.

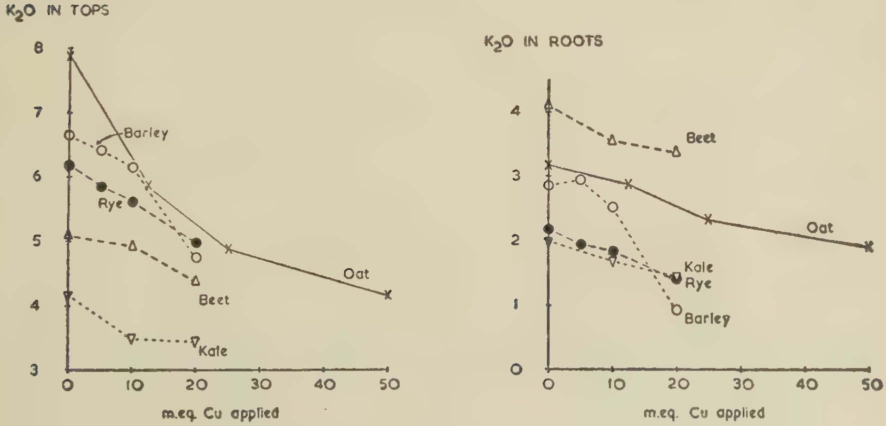


Fig. 7. The effects of copper on potassium status.
Ordinates : % K_2O in dry material.
Abcissae : m.eq. Cu applied (as $CuSO_4$) per pot.

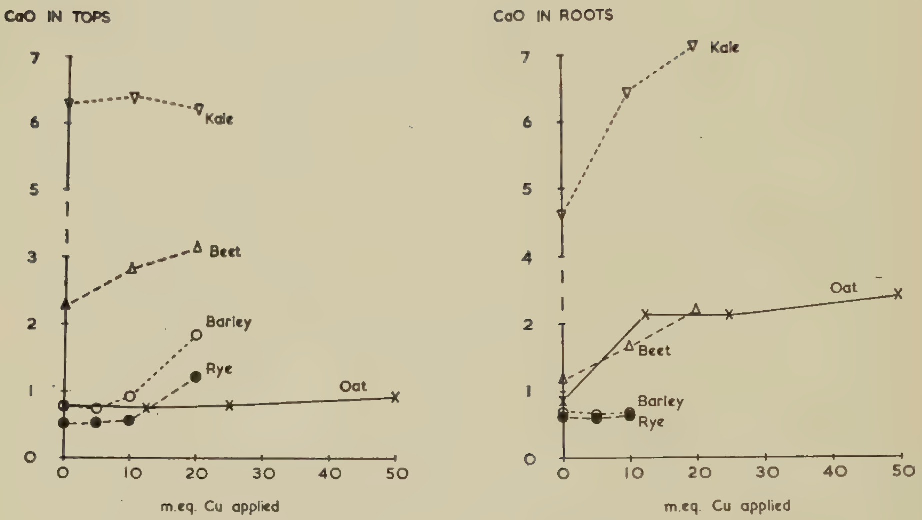


Fig. 8. The effects of copper on calcium status.
Ordinates : % CaO in dry material.
Abcissae : m.eq. Cu applied (as $CuSO_4$) per pot.

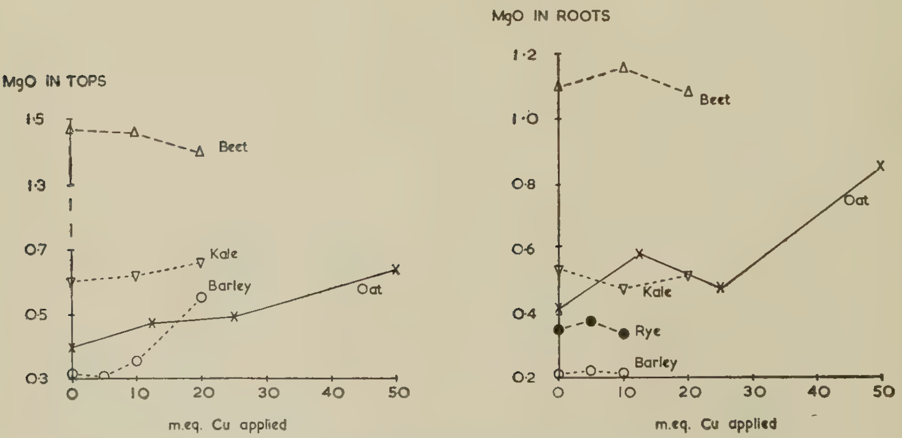


Fig. 9. The effects of copper on magnesium status.
Ordinates : % MgO in dry material.
Abcissae : m.eq. Cu applied (as $CuSO_4$) per pot.

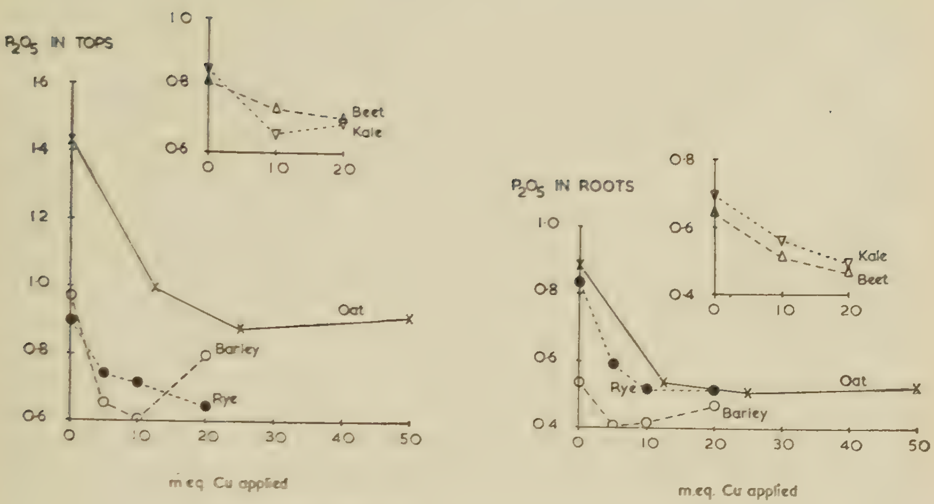


Fig. 10. The effects of copper on phosphate status.
Ordinates : % P_2O_5 in dry material.
Abcissae : m.eq. Cu applied (as $CuSO_4$) per pot.

Summary.

1. Oat, rye, barley, sugar beet and kale plants were grown in soils treated with copper sulphate at various rates.
2. In all crops, Cu application was associated with decreased dry weight, K_2O and P_2O_5 contents, and increased Cu and Mn contents of the crops.
3. Both CaO and MgO status increased with $CuSO_4$ treatment, though in oat and kale, CaO was increased only in roots, not in tops.
4. An increasing supply of copper did not significantly change the Fe status of plant tops.
5. The application of copper to barley and rye produced decreases in dry weights, and increases in copper and manganese status, which were similar in magnitude in the two crops.

Acknowledgments.

I am indebted to Dr. D. J. D. Nicholas for his advice throughout these experiments. Thanks are also due to Mr. G. H. Jones, A.R.P.S., for the photographic records made, and to Mr. D. H. Rees of Rothamsted Experimental Station for advice on the statistical analyses.

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SOME EFFECTS OF METALS IN EXCESS ON CROP PLANTS GROWN IN SOIL CULTURE: VI. EFFECTS OF COBALT AND COPPER ON MINERAL STATUS OF OAT.

By **W. A. FORSTER.**

Paper II in this series described preliminary experiments in which various crop plants were grown in soils treated with copper and zinc sulphates respectively (9). Papers IV and V contained additional information on the effects of copper on these plants (2, 3). The present paper reports the results of a factorial pot experiment in which oat was grown in soil treated with graded levels of cobalt and copper sulphates, both singly and in combination. The two metals when present in excess produced symptoms of iron deficiency and significantly decreased the concentration of iron in the plant tops. They also changed the status of Cu, Mn, K, Ca, Mg and P, and reduced the dry weights of tops.

Experimental.

The experiment consisted of 192 bitumen painted 6 in. pots containing a medium loam of pH 6·8. The treatments were arranged at random in each of six blocks in the glasshouse, each block containing two pots for each of the sixteen treatments. Copper was applied on 10th June at 0, 12·5, 25 and 50 m.eq. per pot respectively. Six oat seedlings (var. S 84) were transplanted on the 13th June into each pot; the seedlings were eight days from seed and about 4 in. high. Three days later cobalt was applied at 0, 4, 7 and 10 m.eq. Co per pot respectively.

The plants were harvested on the 26th June, and the two replicates of each treatment within a block were combined. The plant tops were cut off just above the most mature leaf. Care was taken to avoid soil contamination of the foliage during the course of the experiment and subsequent sampling. The samples were not washed at harvest, but all traces of dust were removed with a camel-hair brush. The plant tops were cut into short lengths and dried for approximately 24 hours at 100° C. in weighed beakers, when they reached constant weight. Dry weights were recorded.

The samples were digested using nitric and perchloric acids (6), and the digests made to 100 ml. Suitable aliquots were taken for the respective chemical analyses. References to the analytical methods used are as follows: copper (10), iron (5), manganese (8), potassium by a cobaltinitrite method, total calcium and magnesium (1), and phosphorus (4).

Results.

Six days after transplanting, a few plants at all rates of copper developed necrosis at the tips of the older leaves: the effect was most marked at the highest rate of copper. The following day, symptoms of iron deficiency appeared in the leaves of several metal-treated plants. The effect was most marked at the highest rate of cobalt, and was similar in degree at all rates of copper and at the two lower rates of cobalt applied. Statistical analyses

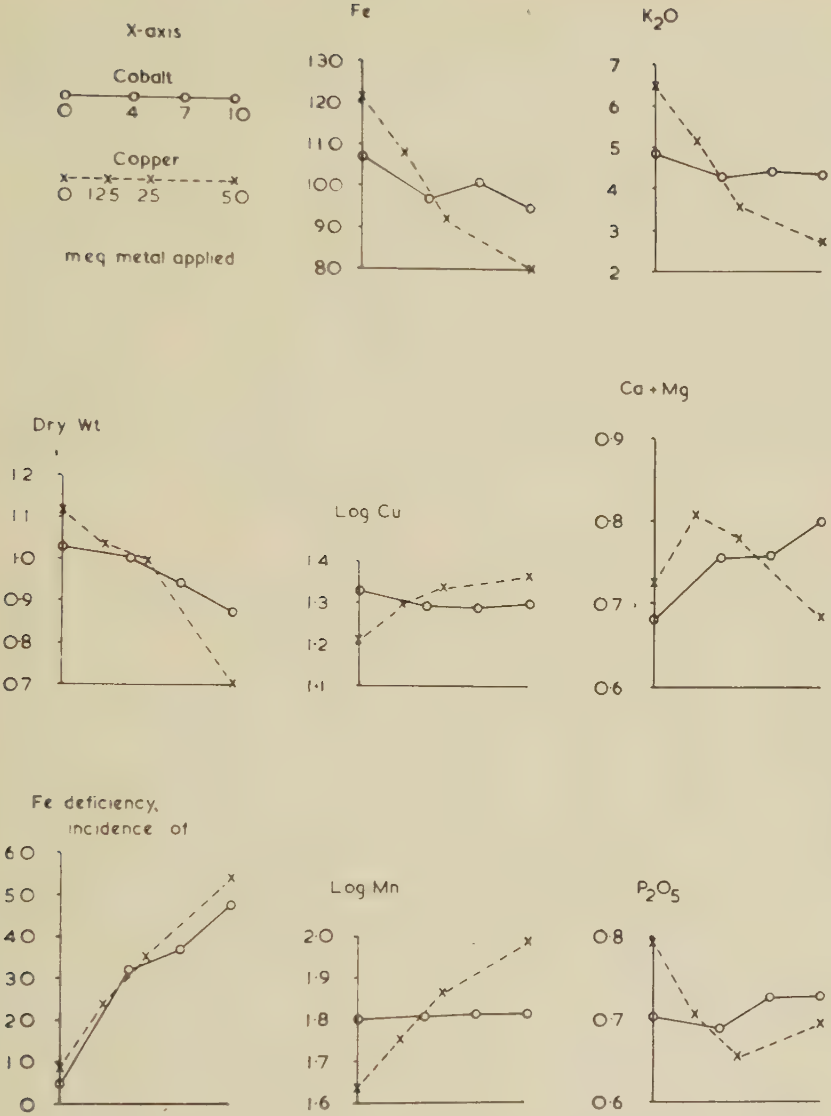

Fig. 1. The effects of cobalt and copper on growth and mineral status of oat.
Abcissae : m.eq. metal per pot applied to the soil.
Ordinates : dry weight — g. per 2 pots.
Fe deficiency — number of plants per 2 pots (in angular degrees) showing symptoms.
Fe, Cu, Mn — log (μg./g. dry material).
K_2O, P_2O_5 — % dry material.
Ca + Mg — m.eq./g. dry material.
Each point is the mean of 24 determinations.

of dry weights, incidence of iron deficiency symptoms at harvest, and chemical analyses are given in Table I. The results are also illustrated in Fig. 1. Fig. 2 shows the interactions found to be statistically significant. The symbols Cu_0,

Co_0, etc., refer to the respective milli-equivalent (m.eq.) rates of metal treatment.

Fig. 1 shows that Cu decreased dry weight and Fe status more than did Co. The effect of metal application on Fe status is also given in Fig. 2, which shows that Fe content is reduced by Cu application at all rates of Co, whereas the significant reduction of Fe status by Co (Table I) is due largely to the decrease occurring at the Cu_2 level.

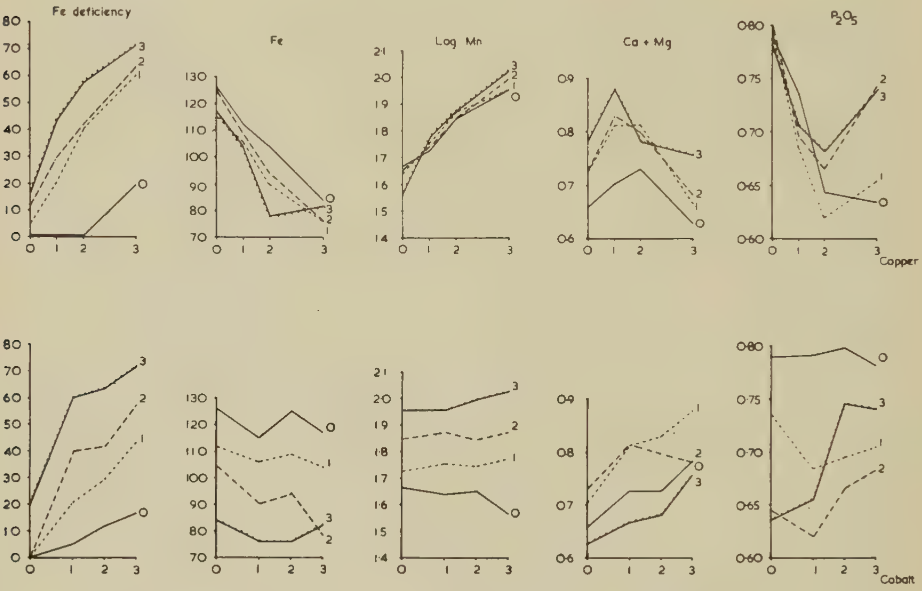

Fig. 2. The effects of cobalt and copper on mineral status of oat.
Upper series : the effect of increasing copper at Co_0, Co_1, Co_2 and Co_3 respectively.
Lower series : the effect of increasing cobalt at Cu_0, Cu_1, Cu_2 and Cu_3 respectively.
Each point is the mean of 6 determinations, the units of the ordinates are the same as in Fig. 1.

The incidence of Fe deficiency symptoms at either Cu_3 or Co_3 (Fig. 1) was high. Fig. 2 shows this to be due largely to a Cu × Co interaction. Thus at Cu_3Co_0 or Co_3Cu_0 only about 20 plants showed symptoms : at Cu_3Co_3 the number increased to 71·4. (20 and 71·4 represent the numbers of plants, in angular degrees, showing iron deficiency symptoms).

There was a significant correlation between the number of plants in each sampling unit showing iron deficiency symptoms, and the iron content of the plant tops. Analysis of covariance gave the following results :—

	D.F.	'r.'	Significance.
Blocks	5	0·568	N.S. ($P > 0·05$)
Treatments	15	—0·825	0·001
Error	75	—0·112	N.S. ($P > 0·05$)
Total	95	—0·693	0·001

'r' is negative, showing that low Fe status is associated with a high incidence of deficiency symptoms, as can be seen in Fig. 1.

Copper status is increased by the application of Cu, but slightly decreased by the application of Co (Fig. 1).

Mn status is greatly increased by the application of Cu to the soil, although apparently unaffected by Co application (Fig. 1). Fig. 2 shows, however, that Co applied in the absence of Cu significantly reduced Mn status.

Potassium content is decreased by the addition of Co, and especially Cu, to the soil. The decrease in K status is similar at all rates of Co applied, but there is a progressive decrease from Cu_0 to Cu_3 (Fig. 1).

The Ca + Mg content is generally higher at Cu_1 and Cu_2 and lower at Cu_3 than at Cu_0, at each level of Co (Fig. 2). The decrease at Cu_3 may be related to the increase in Mn uptake: it has been observed that the application of Mn to oat plants grown in soil led to a decrease of both Ca and Mg contents (7). Fig. 2 shows that the Ca + Mg content was generally increased by the application of cobalt.

Because of the Cu × Co interaction, the effects of Cu and Co on P status are best shown in Fig. 2. In the absence of Co, all rates of Cu applied produced a decrease in P status. The addition of Co counteracts the reduction in P status occurring at Cu_3. At Cu_2, and particularly Cu_3, application of Co is associated with an increase in P content.

TABLE I.

MEAN VALUES, AND THE DIFFERENCES REQUIRED FOR SIGNIFICANCE, AT THE RESPECTIVE RATES OF CU AND CO APPLIED.

Variable	Metal applied	Rate of metal application: 0	1	2	3	L.S.D.
Dry weight of tops (g./2 pots)	Cu	1·12	1·03	0·99	0·70	0·10 at $P = 0·001$
	Co	1·03	1·00	0·94	0·87	0·10 at $P = 0·001$
Fe deficiency symptoms (angular degrees)	Cu	8·4	23·4	34·9	53·7	12·3 at $P = 0·001$
	Co	4·9	31·4	36·7	47·3	12·3 at $P = 0·001$
Fe (μg./g.)	Cu	121	108	92	80	7·9 at $P = 0·001$
	Co	107	97	101	95	7·9 at $P = 0·001$
Cu ($\log_{10}$ μg./g.)	Cu	1·21	1·17	1·34	1·36	0·06 at $P = 0·001$
	Co	1·30	1·26	1·26	1·26	0·03 at $P = 0·05$
Mn ($\log_{10}$ μg./g.)	Cu	1·63	1·75	1·86	1·99	0·06 at $P = 0·001$
	Co	1·80	1·81	1·81	1·81	— N.S. —
K_2O, %	Cu	6·46	5·12	3·55	2·72	0·53 at $P = 0·001$
	Co	4·83	4·28	4·41	4·33	0·40 at $P = 0·01$
Ca + Mg (m.eq./g.)	Cu	0·72	0·81	0·78	0·68	0·04 at $P = 0·001$
	Co	0·68	0·76	0·76	0·80	0·04 at $P = 0·001$
P_2O_5, %	Cu	0·79	0·71	0·65	0·69	0·04 at $P = 0·001$
	Co	0·70	0·69	0·73	0·73	0·03 at $P = 0·01$

Summary.

1. Details are given of a factorial pot experiment in which oat was grown in soil treated with copper and cobalt sulphates, both singly and in combination, at various rates.
2. Copper and cobalt treatments decreased the dry weights of oat tops. They also produced symptoms of iron deficiency in the leaves and a reduction in the total iron status. A significant negative correlation ($P = 0{\cdot}001$) was observed between the number of plants per pot showing iron deficiency symptoms, and their Fe status.
3. Copper and manganese status of oat was increased by the application of Cu. The application of Co reduced the Cu content, but reduced Mn content at Cu_0 only.
4. The sum (Ca + Mg) content was increased, and K content was decreased, by the addition of either Cu or Co. Copper application reduced the P content of oat tops at Co_0, whereas cobalt application at Cu_0 had no effect. At Cu_2 and Cu_3, P status was greater when both Cu and Co were applied together, than when Cu alone was added.
5. The effects of Cu on dry weights, and Cu, Mn, K, Ca, Mg and P status are similar to those observed in previous experiments. In the latter, however, iron deficiency symptoms were not produced, and no relationship was observed between copper application and the Fe status of treated plants.

Acknowledgments.

The author is indebted to Dr. D. J. D. Nicholas for his advice throughout the experimental work described above. Thanks are also due to Mr. D. J. Fisher who made the manganese determinations.

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THE TANNIN OF APPLE JUICE AND CIDER: AN INTERPRETATION OF THE PERMANGANATE TITRATION METHOD.

By A. H. WILLIAMS.

' Although the "tannin" content of apple fruit is an important character, ranking with sugar content and acidity in determining the classification of the fruit for dessert or culinary use or for cider-making, very little is known of its precise chemical nature. "Tannin" is usually estimated by direct titration with potassium permanganate, indigo-carmine being used as indicator and the result calculated in terms of gallotannin (1). It has long been realised that the figure thus obtained is arbitrary, being a measure of permanganate-reducing substances rather than tannins, and that the apple tannins, whatever their precise nature, are not gallotannins but derive rather from catechol (green colour with iron salts, while gallotannins give blue) and resemble in some respects Freudenberg's class of condensed tannins (1), (2).

There does not seem to be any evidence that an appreciable part of the permanganate titration figure is due to substances not of phenolic type. There is, however, much evidence that the titration figure includes a number of phenolic substances of differing types. Part can be precipitated with gelatine, more adsorbed on hide powder, while others are not removed by these reagents. Some workers attempt a distinction between polyphenols and "true tannins" on the basis of this type of fractional precipitation, but since the relative proportions vary according to the precise conditions used such a distinction seems essentially arbitrary.

Only a few phenolic substances have been identified in the apple fruit. The anthocyanin idaein and a quercetin derivative have been isolated from the skin (3), (4), and phloridzin from the pips (5), but these have not been reported in the flesh or juice apart from a colour test identification of quercetin (2). Chlorogenic acid has been identified (6), (7), (8), and the presence of other polyphenols recognised (6), (8) in the flesh and juice.

The polyphenols and tannins (henceforth referred to jointly as phenolics) of apple fruits form a group of substances of very similar properties and it is clear that older methods of chemical separation have met with little success on account of this similarity of properties and because of the sensitivity of the substances involved towards heat, acids and alkalis and oxidation. Paper partition chromatography (9) as applied by Bate-Smith (10), seemed a promising method and has been applied with some success.

Experimental.

Preliminary chromatograms, using Whatman No. 11 and No. 2 papers, showed that the phenolics present in apple juice and cider can be separated into a number of individual components. These were detected by spraying the chromatogram with a formulation of diazotised *p*-nitraniline, which is a general colour reagent for phenols. Some of the compounds also react with a vanillin-hydrochloric acid reagent, while others are visible in ultra-violet light by their fluroescence.

On these thin papers only small quantities of juice or cider (about 10 μl. per spot) can be run; for larger amounts (up to 80 μl. per spot, or 2 ml. on a sheet 18″ × 22″) a thick paper, Whatman No. 3MM was used. By inspection in ultra-violet light and by spraying strips from the sides of the sheets with the colour reagents it is possible to cut up the large sheets into zones each containing one or more of the various phenolics. These compounds can be estimated approximately by immersing the paper carrying the various zones in the solution (0·5 ml. N H_2SO_4 in 200 ml. water) used for the standard permanganate method and titrating the eluted phenolics. An appropriate choice of solvents makes it possible to separate several of the individual phenolics in turn; the most useful solvents have been: *sec*-butanol-acetic acid-water (69:2:29), phenol (saturated with water and equilibrated with an equal volume of 2% acetic acid), and plain water.

Although it has been found that juice expressed from the apple with adequate precautions to prevent enzymic oxidation—i.e. addition of sulphur dioxide to the pulp, rapid separation of the juice and inactivation of the enzymes by heating—presents a rather clearer picture on the chromatogram, it was felt that for the interpretation of the permanganate titration it was preferable to follow more closely the normal conditions of pressing. A high-tannin variety of cider apple, Tardive Forestier, was chosen for the main experiments. No evidence of significant change in the phenolics during fermentation was found, so most of the experiments were made on a dry cider from this variety to avoid difficulties due to the overloading of chromatograms by the large amounts of sugars present in unfermented juice.

Some other varieties of apples have been examined qualitatively and show a similar pattern of phenolics to that found for Tardive Forestier. Differences are generally due to variations in the amounts of the various substances rather than in their presence or complete absence.

Results.

The nature of the phenolics. The most obvious phenolic in apple juices and ciders is chlorogenic acid; in low-tannin varieties it is the only individual substance clearly identified. Chromatograms from cider varieties show in addition a strong spot corresponding to epicatechin, and another corresponding to catechin; this latter spot ranges from an intensity approaching that of the epicatechin spot, in Yarlington Mill and Upright Medaille d'Or, to very weak in Tardive Forestier and Dabinett.

In addition to these there are several as yet unidentified spots whose colour reactions suggest that they belong to the class of compounds described by Robinson as leuco-anthocyanins (11) (12). They are most clearly recognised in the phenol-acetic acid solvent, where they form a series of at least four spots merging into a phenolic trail stretching back to the origin.

Approximate R_F values in the solvents used for those substances recognisable as individuals are given in Table I. It is to be stressed that these values are approximate and apply only to the conditions of experiment used, for it is found that in complex mixtures like ciders, containing other substances such as acids and carbohydrates beside the phenolics, the R_F values can vary considerably according to the exact running conditions and loading of the chromatograms.

TABLE I.

APPROXIMATE R_F VALUES OF APPLE PHENOLICS.

	Water	Phenol/acetic acid/water	sec-Butanol/acetic acid/water
Chlorogenic acid ..	0·78	0·56	0·46
Catechin	0·29	0·46	0·66
Epicatechin	0·22	0·46	0·54
Leuco-anthocyanin ..	0·46	0·34	0·34
,, ,, ..	0·34	0·23	0·21
,, ,, ..	—	0·17	—
,, ,, ..	—	0·10	—

Estimation of the individual components. For the running of sheets of thick paper to be cut into zones for permanganate titration of the different phenolics, water and the phenol solvent were found to give the best separation. The sheets were cut into zones corresponding to the recognised phenolics as far as possible, the numbering beginning from the point at which the cider was applied to the chromatogram. The percentages of the total titration found in the various zones are shown in Table II and Table III, first column.

TABLE II.

SEPARATION OF PHENOLICS IN PHENOL/ACETIC ACID/WATER SOLVENT.

Zone number	Description	Percentage total titration
1	Point of application, and trail containing leuco-anthocyanin. 1 and 2 arbitrarily divided; 3 and 4 represent peaks of intensity	10·2
2		5·3
3		7·4
4		6·4
5	Gap, no phenolics	0·0
6	Leuco-anthocyanin	6·4
7	Gap, little phenolic	2·5
8	Leuco-anthocyanin	13·1
9	Gap, no phenolic	0·3
10	Epicatechin	16·6
11	Chlorogenic acid	30·0
12	Solvent front	1·1

The colour reagents showed that zones 5, 7, 9, and 12 contained but little of phenolic nature. Zones 1–4 showed a continuous phenolic "streak" with the reagents; the division of zones 1 and 2 was arbitrary, but zones 3 and 4 showed definite peaks in the trail with leuco-anthocyanin colour reaction.

TABLE III.

SEPARATION OF PHENOLICS IN WATER SOLVENT.

Zone No.	Description in untreated cider	% total titration of untreated cider		
		(1) Untreated	(2) Gelatine treated	(3) Hide powder treated
1	Point of application : arbitrary division, little phenolic present	1·7	0	0
2		4·5	0	0
3		4·5	0	0
4	Epicatechin	21·5	13·1	1·5
5	Leuco-anthocyanin	15·8	9·2	0
6	Gap, little phenolic	0·6	0	0
7	Leuco-anthocyanin	10·2	6·2	1·5
8	Gap, little phenolic	3·4	1·6	1·5
9	Chlorogenic acid	33·3	26·2	8·5
10	Solvent front	4·5	1·6	0·7
	Total	100	57·9	13·7

Zones 1, 2, 3, 6, 8, 10 were shown by the colour reagents to contain only a small part of the phenolic substances, and some of the material present is due to overlap from other zones. Zone 7 corresponds to zone 8 of Table II, and zone 5 to zone 6 of Table II. Zones 4 and 5 probably contain in addition other leucoanthocyanins which occur in zones 3 and 4 of the phenol sheets.

The effect of the treatment with hide powder and gelatine is shown in Table III, columns 2 and 3. The phenolics remaining after treatment are expressed as a percentage of the original permanganate titration of the untreated cider.

Specificity of the permanganate titration. All the phenolics so far identified in apple juice contain the catechol grouping. Clearly the permanganate titration as used estimates these compounds, so a number of other types of phenolic substances were tested under the conditions of the permanganate titration. Some did not react; for others the oxygen taken up for their titration was calculated. The results, expressed as atoms of oxygen per molecule of phenolic, are set out in Table IV.

TABLE IV.

PERMANGANATE OXIDATION OF PHENOLS.

	Atoms O per mol.
Catechol	2·3
Resorcinol	0
Quinol	2·5
p-Hydroxybenzoic acid	0
2:4-Dihydroxybenzoic acid	0
3:4 ,, ,,	2·3
2:5 ,, ,,	2·3
Phloroglucinol	0
Pyrogallol	2·9
Gallic acid	3·0
Tyrosine	0
3:4-Dihydroxyphenylalanine	2·8
Phloridzin	0
Catechin	2·2
Caffeic acid	3·3
Chlorogenic acid	3·3
Quinic acid	0
Cinnamic acid	0·9

Discussion.

The results presented in Table IV show that only phenolics containing the ortho- or para-dihydroxybenzene structures in some form react under the conditions of the permanganate titration. Monohydric phenols, or those phenols with the meta-dihydroxybenzene structure, do not react. This behaviour is in accordance with the generally recognised sensitivity of the ortho- and para-dihydric phenols to oxidation.

Those compounds whose only active grouping is of the catechol type take up about 2·3 atoms of oxygen per molecule in the permanganate titration. Pyrogallol types take up rather more, about 3 atoms per molecule. Caffeic and chlorogenic acids, which might be expected to follow the catechol type, take up still more—3·3 atoms per molecule. This is apparently due to their being cinnamic acid derivatives ; possibly the unsaturated side chain undergoes partial oxidation, for cinnamic acid itself takes up 0·9 atoms of oxygen per molecule in the permanganate titration, which when added to that for 3:4-dihydroxy-benzoic acid (2·3) can account for the caffeic acid figure of 3·3. The quinic acid moiety of chlorogenic acid clearly makes no contribution to its titration.

Tables II and III show that the total tannin content of the cider examined can be divided into at least four and probably six components ; chlorogenic acid, epicatechin and at least two and probably four substances with leuco-anthocyanin reactions. Together these six components account for about 80% of the total tannin titration. The remainder shows leuco anthocyanin reactions and is probably of that type.

It is evident from the figures given in Table III that precipitation by gelatine and hide powder cannot be made the basis for a rigid distinction between " true tannins " and polyphenols. Precipitation by gelatine is selective in the sense that the unidentified and presumably more complex phenolics are more readily removed than the comparatively simple chlorogenic acid and epicatechin, but the removal is essentially progressive and depends on the amount of gelatine added. Hide powder, on the other hand, removes the major part of the simple polyphenols as well as the bulk of the other compounds. It seems that any attempt at a clear-cut distinction between " tannins " and polyphenols is bound to be arbitrary and dependent on the nature of the particular reagent used. The behaviour towards gelatine and hide powder and the results of solvent extraction experiments on the same cider, taken in conjunction with the similarity of chemical reactions of the phenolics involved (other than chlorogenic acid) suggest a gradation of properties which makes such attempts pointless.

Summary.

The permanganate titration method for the estimation of " tannin " has been shown to be specific for ortho- (catechol) and para- (quinol) polyphenols. Of these, only phenols of catechol type have been found in apple juice and cider (the phloroglucinol part of catechins and leuco-anthocyanins will not be estimated, the hydroxyls being meta-). Chlorogenic acid, catechins and four compounds which appear to be leuco-anthocyanins have been recognised by paper chromatography and an estimate made of their relative contributions to the total permanganate " tannin " figure in a sample of Tardive Forestier cider.

Acknowledgments.

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AUTHOR INDEX
TO PAPERS IN THE LONG ASHTON ANNUAL REPORTS FROM 1907 TO 1952.

By H. S. D. Swan

The first report on the work at Long Ashton, covering the years 1903-04, was published by F. J. Lloyd, B. T. P. Barker and J. Ettle in the *Journal of the Bath and West and Southern Counties Society* (4th series) vol. **15,** 152-173. This article was reprinted as an Appendix (pp. 9-31) to the Report of the Governors of the National Fruit and Cider Institute, made on 1st June, 1905, during the Bath and West Show at Nottingham. The Institute's work during 1905 was similarly described by Lloyd, Barker and Ettle in the *Bath and West Journal,* **16,** 136-160, which was reprinted as pp. 8-32 of the 1906 Report of the Governors of the N.F.C.I. The Institute's report for 1906, written by Barker and Ettle, appeared in the *Bath and West Journal* (5th series), **1,** 162-180, being reprinted in the 1907 Report of the Governors as pp. 10-40.

The report on the work for 1907 was the first to be issued as a separate publication, which has since continued annually. Up to 1912 it was entitled : "National Fruit and Cider Institute, Long Ashton, near Bristol, Report for the Year 1907" (etc.). In 1913, in consequence of the changed status of the Institute, the title became "The Annual Report of the Agricultural and Horticultural Research Station (the National Fruit and Cider Institute), Long Ashton, Bristol." In addition to the yearly Reports, a special report was issued in 1911 entitled : "Report on the Work of the National Fruit and Cider Institute, 1903-10."

The Author Index and Subject Index following include the titles of papers published in all the Annual Reports from 1907 to 1952 and in the special report for 1903-10 (indicated in the index as 1911*). In the Author Index, the publications, including joint papers, of any one author are listed chronologically. In the column headed 'Year' the date given is that on the title page of the Annual Report in which the paper appears. The date of publication is one year later.

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By H. S. D. SWAN

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